The interrelation of carbon and water balance in beech-dominated forests - from leaf level water use efficiency to stand and area scale assessments

Dissertation

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"'In den Wäldern sind Dinge, über die nachzudenken,
man jahrelang im Moos liegen könnte. "'
Franz Kafka

Contents

Abbrevations

A	photosynthesis, assimilation
A_{max}	CO_2 saturated maximal photosynthetic uptake
ANOVA	analysis of variance
C	carbon
c_a	ambient CO_2 concentration
c_c	CO_2 concentration at the sites of carbon fixation
c_i	intercellular CO_2 concentration
CO_2	carbon dioxide
$\delta^{13}C$	carbon isotopic composition relative to international standard V-PDB
$\Delta^{13}C$	discrimination against ^{13}C
$\delta^{18}O$	oxygen isotopic composition relative to international standard VSMOW
$\Delta^{18}O$	discrimination against ^{18}O
d	days
DBHi	diameter at breast height increment
DGVMs	dynamic global vegetation model
DSD	drought stress dose
ϵ_e	equilibrium fractionation
ϵ_k	kinetic fractionation
ϵ_{wc}	equilibrium fractionation factor for oxygen
E	leaf transpiration
ETR	electron transport rate
G	radiation
g_m	mesophyll conductance
g_s	stomatal conductance
h	hour
H_2O	water
L	effective pathlength of water movement
MRT	mean residence time
NSC	non structural carbohydrates
NRMSE	normalized root mean square error
O	oxygen
Ψ_s	soil water potential
P	precipitation
pET	potential evapotranspiration

PPFD	photosynthetic photon flux density
REW	relative extractable soil water
rH	relative humidity
RubisCO	ribulose 1,5-bisphosphate carboxylase oxygenase
S_Γ	substrate specificity factor
SD	standard deviation
θ_s	volumetric soil water content
T	temperature
TDR	time domain reflection
TLS	terrestrial laser scanning
V_{max}	max photosynthetic capacity
VPD	Vapor Pressure Deficit of air
VPDB	Vienna Pee Dee Belemnite
VSMOW	Vienna Standard Mean Ocean Water
WC	water content
Wd	water deficit
WSOM	water-soluble organic matter
WUE	water-use efficiency
WUE_i, iWUE	intrinsic water-use efficiency
ww	well watered
XWF	xylem water flow model
y	years

Summary

Current climate models predict that many important forest regions in Central Europe will experience increasing frequencies and severities of drought periods. Beech-dominated forests are an important part of these forest regions. Shortage in water supply is one of the most important limiting factors for growth of plants and thereby linked to a reduced carbon gain. Another key stressor is the competition between as well as within a plant communities. Due to this fact it is important to understand the plants physiological mechanisms during drought as well as inter-specific and intra-specific competition in beech dominated forest ecosystems. This present study documents the interrelation of carbon and water balance and the interactions of its pathways during drought on different spatial (leaf area to the stand level) and temporal scales (intra-annual to decadal). Six relevant species were selected in total (tree species: *Fagus sylvatica*, *Acer platanoides*, *Fraxinus excelsior* and species from the understorey of beech dominated forests: *Impatiens noli tangere*, *Mercurialis annua* and *Allium ursinum*). All of them have developed various strategies to cope with competition and avoid or tolerate drought stress. Depending on the drought intensity (e.g. moderate realistic drought) small effects in mesophyll conductance (g_m), intrinsic water use efficiency (iWUE) and amount of new assimilates within trees occurred. Theses effects were completely reversible. In chapter **I** different short-term strategies modifying the interrelation between water loss and carbon gain during drought are shown at the leaf level. Thereby, responses of mesophyll conductance (g_m) and effective pathlength of water movement in the leaf (L) were examined in response to soil moisture availability for seedlings of six species using oxygen and carbon isotope techniques combined with gas exchange measurements (A/c_i curves) and chlorophyll fluorescence techniques. In chapter **II** a ^{13}C-pulse labeling greenhouse study focus on medium-term allocation patterns of carbon into/through different plant tissues under moderate drought and competition at the single tree level. This study was performed with two year-old broadleaf *Fagus sylvatica* and *Acer platanoides* trees. Based on a retrospective tree-core analysis from 2002 to 2007, in mature (>80y) beech trees the effects of the 2003 drought on growth and physiology are discussed in chapter **III**. This field study contains tree ring increment analyses, xylem water flow modelling (XWF) and intrinsic water use efficiency calculations (iWUE). The study in chapter III highlights the importance of acclimation to drought stress in beech trees and points out several uncertainties of its determination. A review study is also included and deals with isotope mixing effects e.g. during drought and scales up from the molecule to the forest stand level. This PhD-study provides information on the following research questions in

detail.

1. The leaf level: Are the pathways for CO_2 and water movement in the leaves linked in an inverse way and both affected by drought? Does the tortuosity for leaf water movement (L) scales inversely with g_m in different herbaceous and tree species in a beech dominated forest ecosystem? Does a drought increase in g_m not only positively affect carbon assimilation but also feed back on leaf hydraulic properties (e.g. L)?

2. The whole plant level: Does drought affect the C transport within the plant and are drought responses mediated by species interaction? Leads moderate drought in general to a reduction of assimilate transport belowground and thus to a lower ^{13}C signal in soil respired CO_2 after $^{13}CO_2$ pulse labelling? Moreover, is Norway maple able to outcompete the drought sensitive beech under water restriction mainly by taking advantage of the impairment of beech to allocate newly assimilated carbon belowground?

3. The stand level: Caused the limited soil water supply within the year 2003 a reduction of the annual radial growth mainly because of an efficient mechanism of stomatal control upon soil water restriction leading also to an increased iWUE?

Contrary to our expectation the results regarding task **1** indicate that an increase in g_m does not automatically imply a reduction in L, and subsequently not in the tortuosity of the water movement in the leaf lamina either. We found no clear common response in g_m towards drought for the three selected tree species: European beech decreased, Norway maple increased and common ash showed a constant g_m in the drought compared to the well watered treatment. The missing relationship between g_m and L points either to different main pathways for CO_2 and water movement in the mesophyll or to different regulation of a common pathway. In terms of task **2** we observed that moderate drought reduced photosynthesis rates in both species with a stronger effect to beech. Both species maintained their assimilate transport to the belowground compartment. The experiments in chapter II showed that the European beech increased the relative amount of newly assimilated C in roots during drought and under inter-specific competition. Competition had more influence on changes in carbon allocation than moderate drought. Furthermore, we point out that European beech is still a strong competitor during drought plus inter-specific competition. The key message of task **3** is that harmful effects of water shortage in the dry and hot summer 2003 has been avoided by an effective stomatal closure while use of carbon storage pools (remobilization) may have prevented carbon starvation and growth reduction. In detail, the functional-structural single tree modeling (XWF) approach applied, revealed a strong limitation of water use and carbon gain during drought. However, tree ring width and isotope data did not show a clear drought response in isotope based iWUE and no differentiation in radial growth

during six subsequent years (2002 to 2007) has been observed. We used integrated results from mechanistic carbon-water balance simulations, tree ring carbon and oxygen isotope analysis and tree ring width measurements, we postulate that the suggested drought induced growth decline has been prevented by the remobilization of stored carbohydrates, an early onset in growth and the relatively late occurrence of severe drought in 2003. We also need to consider that the isotope signal in the tree-core cellulose might be influenced by isotope mixing processes or other plant physiological reactions. Thereby, it is still not clear if the mature beech trees really suffered from drought stress at the Tuttlingen site in 2003.

It seems, starting at the leaf area scale up to the individual single tree level, that a realistic moderate drought as it is occurring normally and periodically in forest ecosystems, is completely reversible and can be easily tolerated by the different species tested. Moreover, even a strong drought event e.g. 2003 might not be necessarily, depending on timing, duration of drought and on the tree C status (the availability of storage pools) of the years before and after the drought. It is, however, still not clear how different processes controlling the C and water balance are coordinated on different spatial and temporal scales. Further work is necessary to bridge the mechanisms acting on various scales, especially the scaling up from the single tree level to the forest stand level needs to be improved.

Zusammenfassung

Aktuelle Klimamodelle prognostizieren, dass viele bedeutende Waldregionen in Mitteleuropa zukünftig einer steigenden Frequenz und höheren Intensität von Dürreperioden ausgesetzt sein werden. Buchendominierte Wälder bilden einen wichtigen Bestandteil dieser Waldregionen. Der Mangel hinsichtlich der Wasserversorgung ist eine der wichtigsten limitierenden Faktoren für das Wachstum der Pflanzen sowie der damit verbundene reduzierte Zugewinn an Kohlenstoff. Ein weiterer bedeutender Stressfaktor ist die Konkurrenz zwischen sowie innerhalb Pflanzengemeinschaften. Aufgrund dieser Tatsache ist es wichtig, die pflanzenphysiologischen Mechanismen während der Trockenheit sowie interspezifische und intra-spezifische Konkurrenz in buchendominierten Waldökosystemen zu verstehen.

Die vorliegende Arbeit untersucht den Zusammenhang von Kohlenstoff- und Wasserhaushalt und deren Wechselwirkungen sowie die Transportwege während der Trockenheit auf unterschiedlichen räumlichen (Blattebene bis Bestandesebene) und zeitlichen (kurzfristig bis langfristig) Skalen. Insgesamt wurden sechs Arten ausgewählt (*Fagus sylvatica*, *Acer platanoides*, *Fraxinus excelsior*, *Impatiens noli tangere*, *Mercurialis annua* und *Allium ursinum*). Alle Arten haben verschiedene Strategien entwickelt, um Konkurrenz und Trockenstress zu bewältigen, zu verhindern oder zu tolerieren.

In Abhängigkeit von der Trockenstressintensität wurden geringe Effekte, in der Mesophyllleitfähigkeit (g_m), intrinsische Wassernutzungseffizienz (iWUE) sowie in der Transportmenge an neuen Assimilaten beobachtet. Diese beobachteten Effekte sind vollständig reversibel. In Kapitel I wurde gezeigt, dass kurzfristig auf der Blattebene verschiedene Mechanismen, die Wechselbeziehung zwischen Wasserverlust und Kohlenstoffertrag während Trockenstress modifizieren. Reaktionen wurden in der Mesophyllleitfähigkeit und der effektive Weglänge der Wasserbewegung (L) für Setzlinge von sechs verschiedenen Arten, unter Verwendung von Sauerstoff- und Kohlenstoffisotopenanalysen, A/c_i Kurven sowie chlorophyll-fluoreszenz Messungen nachgewiesen. Kapitel II fokussiert eine ^{13}C-Pulsmarkierung im Gewächshaus sowie deren zeitlich mittelfristige Allokation von Kohlenstoff in und durch die verschiedene Pflanzengewebe bezüglich moderatem Trockenstress und Konkurrenz zwischen den Bäumen. Diese Studie wurde an zweijährigen Buchen (*Fagus sylvatica*) und Ahorn (*Acer platanoides*) durchgeführt. Basierend auf einer retrospektiven Baumkernanalyse von 2002 bis 2007, in alten Buchenbeständen (>80 Jahre), wurden in Kapitel III die Auswirkungen der Trockenheit (speziell im Jahr 2003) im Wachstum und auf pflanzensphysiologische Reaktionen untersucht. Diese Feldstudie enthält Baumzuwachsanalysen, Xylem-Wasserdurchfluss-

Modellierung (XWF) und Berechnungen hinsichtlich intrinsischer Wassernutzungseffizienz (iWUE). Die Studie in Kapitel III unterstreicht die Bedeutung der Anpassung der Buche an Trockenheit und führt Unsicherheiten in der Quantifizierung der oben aufgezählten Parameter an. Eine Überblicksstudie ist ebenfalls enthalten, welche sich mit Isotopenmischeffekte beschäftigt. Dieser Review skaliert von der molekularen Ebene auf die Bestandesebene. Die vorliegende Doktorarbeit gibt Auskunft über die folgenden Fragestellungen im Detail.

1. Blattebene: Sind die Wege für CO_2- und Wassertransport in den Blättern invers miteinander verbunden und werden beide durch Trockenheit beeinflusst? Korreliert die Tortuosität für die Blattwasserbewegung (L) invers zu g_m in verschiedenen Pflanzengemeinschaften (Bäume, Sträucher) in einem buchendominierten Ökosystem? Induziert eine Trockenheit die Zunahme von g_m und stellen sich positive Effekte auf Kohlenstoffassimilation und blatthydraulische Eigenschaften ein (z.B. L)?

2. Gesamte Pflanze: Beeinflusst Trockenheit den Kohlenstofftransport innerhalb der Pflanze? Werden pflanzenspezifischen Reaktionen bezüglich Trockenstress in Form von Wechselwirkungen zwischen den Arten gesteuert? Führt moderate Trockenheit im allgemeinen zu einer Verringerung des Assimilattransportes in den Unterboden und somit zu einer niedrigeren Bodenrespiration? Ist Spitzahorn im Gegensatz zur trockenempfindlichen Buche in der Lage neue Assimilate effizienter in den Unterboden zu verlagern? Könnte diese Konkurrenzfähigkeit zur Verdrängung der Buche beitragen?

3. Bestandesebene: Reduziert die begrenzte Bodenwasserversorgung im Jahr 2003 das jährliche radiale Wachstum, vor allem durch eine effektive stomatäre Leitfähigkeit und resultiert daraus eine Erhöhung in iWUE?

Entgegen unserer Erwartung bezüglich Fragestellung **1** zeigen die Ergebnisse, dass eine Erhöhung in g_m nicht automatisch eine Verringerung in L mit sich bringt sowie auch nicht in der Tortuosität der Wasserbewegung in der Basalmembran. Es zeigt sich keine klare Reaktion von g_m in Abhängigkeit von der induzierten Trockenheit gegenüber der gut bewässerten Kontolle. Beispielsweise veränderte sich g_m für die ausgewählten Baumarten folgendermaßen: Buche zeigt eine verringerte , Ahorn eine erhöhte und Esche eine konstante g_m. Die fehlende Beziehung zwischen g_m und L deutet entweder auf unterschiedliche Wege für CO_2 und Wasser im Mesophyll oder auf einen unterschiedlichen Regulierungsmechanismus eines gemeinsamen Weges hin. In Bezug auf Fragestellung **2** wurde beobachtet, dass moderater Trockenstress die Photosyntheseraten bei beiden Spezies verringert, mit einem stärkeren Effekt bei Buche. Beide Arten zeigten keine Beeinflussung in ihrem Assimilattransport in den Unterboden sowie keine zeitlich signifikante Abweichung zwischen den Behandlungsvarianten. Die Experimente in Kapitel

II zeigten, dass Buche die Assimilatmenge (C) in den Wurzeln relativ während der Trockenheit und interspezifischer Konkurrenz erhöht. In diesem Fall hatte Konkurrenz mehr Einfluss auf die Veränderungen der Kohlenstoffallokation als moderate Trockenheit. Darüber hinaus weisen wir darauf hin, dass die Buche immer noch eine dominante Stellung (konkurrenzfähig) während Trockenheit und interspezifischer Konkurrenz besitzt. Die Kernaussage der Fragestellung **3** ist, dass eine schädliche Wasserknappheit durch wirksame stomatäre Reaktionen vermieden werden kann sowie die effiziente Verwendung von gespeichertem Kohlenstoff (Remobilisierung) das Absterben und eine Wachstumsreduktion verhindern können. Im Detail wurde eine Modellierung basierend auf einem Einzel-Baum-Ansatz (XWF) angewendet. Es zeigt sich eine starke Einschränkung im Wasserverbrauch und in dem Zugewinn an Kohlenstoff während der Dürre im Jahr 2003. Allerdings haben die analysierten Jahrringbreiten und Isotopenanalysen keine klare Antwort hinsichtlich Radialwachstum und iWUE unter Dürre während der sechs folgenden Jahren (2002 bis 2007) gezeigt. Erzielt wurden die Ergebnisse mit Hilfe mechanistischer Kohlenstoff-Wasser-Simulationen (XWF), Baumring-Isotopenanalyse hinsichtlich Kohlenstoff und Sauerstoff sowie Jahrringmessungen. Wir postulieren, dass durch die Trockenheit und der damit induzierte Wachstumsrückgang durch die Remobilisierung von gespeicherten Kohlenhydraten verhindert wurde. Dazu beigetragen hat sicher auch, dass die schwere Dürre im Jahr 2003 relativ spät in der Wachstumsperiode aufgetreten ist. Aber berücksichtigt werden muss auch, dass das Isotopensignal aus der Baum-Cellulose durch Isotopenmischprozesse von anderen pflanzenphysiologischen Reaktionen beeinflusst worden sein kann. Dadurch ist unklar, ob die untersuchten Buchen wirklich Trockenstress am Standort Tuttlingen im Jahr 2003 hatten.
Es scheint vermutlich, beginnend auf der Blattebene bis hin zum einzelnen Baum, dass ein realistischer moderater Trockenstress, wie es ist in der Regel periodisch in Waldökosystemen auftritt, vollständig reversibel und leicht durch die verschiedenen getesteten Arten toleriert werden kann. Selbst ein starkes Dürreereignis (wie z.B. 2003) muss nicht immer zwingend schädlich sein. Dies ist vom Zeitpunkt und der Dauer sowie vom aktuellen C-Status des Baumes (wieviel Speicherstoffe verfügbar sind), in den Jahren vor und nach der Dürre, abhängig. Es ist jedoch weiterhin unklar, wie die verschiedenen Prozesse genau den C-und Wasserhaushalt steuern und auf unterschiedlichen räumlichen und zeitlichen Skalen agieren. Weitere Untersuchungen sind notwendig, um die Mechanismen auf verschiedenen Skalen zu übertragen, vor allem die Skalierung von der Einzelbaumebene auf die Waldbestandesebene muss verbessert werden.

Background

There is increasing information that plants and plant communities react in multiple ways of interaction to the predicted changes in climate [Parmesan and Yohe (2003)]. However, there is a lack of knowledge about mechanisms and the plant physiological basis of most of these reactions [Rennenberg et al. (2006)]. Particularly the combined effects including competition on the ecosystem level prevent accurate predictions about the development of carbon sink strength, tree growth and stand development of forests under a changing climate in the future [Malhi et al. (1999)]. Due to the fact that these changes in climate are related to plant physiology, nutrient availability in plants, to the root system reducing growth and vitality, as well as social and economic gain and services provided by forests, thus one of the aims of this study is to present and discuss the multidisciplinary approach among different scales.

On the leaf level scale, the balance between CO_2 uptake and water loss appeared to be well understood [Field et al. (1983), Farquhar and Richards (1984), Warren et al. (2001)]. However, it has been suggested only recently that the potential influence of leaf mesophyll conductance (g_m) on the relationship between transpiration (E) and photosynthesis (A) is important for optimization of water use efficiency of competing plant species [Warren and Adams (2006), Aranda et al. (2007), Barbour et al. (2010), Flexas et al. (2013)]. Furthermore, few articles have shown that not only C assimilation is reduced [Epron et al. (2012), Bahn et al. (2013)] but that also the transfer of newly produced assimilates within trees and from trees to the soil is strongly retarded in drought exposed compared to well watered trees [Ruehr et al. (2009), Barthel et al. (2011), Zang et al. (2014)]. In trees, the interplay between reduced carbon assimilation and the inability to transport carbohydrates to the sites of demand under drought might be one of the mechanisms leading to carbon starvation. On the stand level and forest ecosystem scale, it is well known that the carbon balance is strongly affected by the availability of water or due to drought induced tissue damages decreasing their functionality and requiring additional carbon expenses for repair [Palacio et al. (2014)]. For example, during the dry summer in 2003, a strong decrease in the CO_2 sink strength of forest ecosystems was observed all over Europe [Ciais et al. (2005)]. However, there is not much data on how such drought events affect tree and ecosystem internal C fluxes and how they interact with net ecosystem carbon exchange because of their limited occurrence under past and actual climate [Beniston and Innes (1998)]. In particular for European Beech (*Fagus sylvatica*), which is widespread in central Europe, growth decline has been demonstrated at various sites [Löw et al. (2006), Jump et al. (2006), Granier et al. (2007), Van der Werf

et al. (2007), Charru et al. (2010), Maxime and Hendrik (2011)]. Two main hypotheses have been put forward to explain the reduction of tree growth and increased mortality: carbon starvation and hydraulic failure [McDowell et al. (2008a)]. Carbon starvation summarizes the situation when the carbon demand for maintenance of cellular and defensive metabolism is not met owing to low carbohydrate supply from photosynthesis and storage [McDowell (2011)]. Carbon starvation is expected to occur during prolonged drought periods in relatively isohydric plants closing their stomata at low xylem water tensions, hydraulic failure is expected to proceed more rapidly especially in relatively anisohydric plants keeping their stomata open during drought [Sevanto et al. (2014)]. Moreover, hydraulic failure, seems to be the primary cause of plant mortality during drought due to xylem embolisms [Anderegg et al. (2012), Choat et al. (2012)] but be might avoided under high availability of mobile soluble carbon compounds [Adams et al. (2009), McDowell (2011), Gruber et al. (2012), Sevanto et al. (2014)]. The question, how the impact on tree metabolism can be described considering a simultaneously reduced CO_2 uptake is still open [Sala et al. (2010)].
Tree species-specific variations in the mobilization of non structural carbohydrates (NSC) in response to water shortage and critical thresholds for C storage in trees plays also an important role for the actual C supply during drought [Palacio et al. (2014)]. Moreover, competitive interaction between species with different strategies to withstand drought and with different drought sensitivity might result in even more pronounced negative effects of water availability reduction for sensitve species [Fotelli et al. (2009)].
As a consequence of the knowledge gaps, the present studies aims to contribute to the understanding of plant physiological mechanisms affecting the key parameters of leaves, tree and ecosystem carbon and water balance and of forest growth taking into account the linkage between the different scales (see Fig.1).

Stable isotopes in ecosystem research

Natural isotope abundances

Isotopes are variants of a particular chemical element which differ in neutron number. The isotopes which have the smaller neutron numbers are the light isotopes (e.g. ^{12}C, ^{16}O) and with the greater neutron number are the heavy isotopes (e.g. ^{13}C, ^{18}O). Differences in the natural isotope abundances between different molecules and between educts and products of chemical reactions are caused by isotope fractionation reactions. Frac-

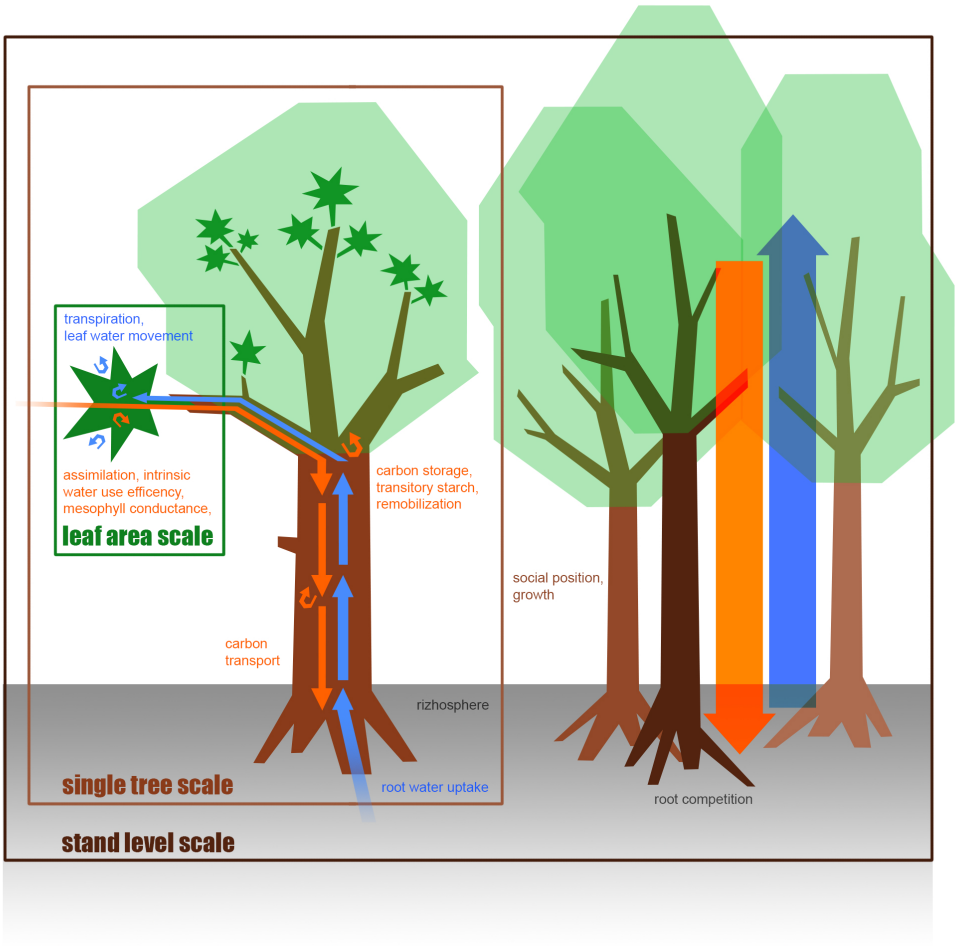

Figure 1: Linkage of carbon and water interrelations from the leaf area scale to the stand level.

tionation means that one isotope is preferred against another isotope during chemical and physical processes. In a closed system the naturally isotopic distribution adapts to an equilibrium fractionation, which is mostly dependent on temperature. Kinetic fractionation can take place in open systems and are expressed in irreversible reactions when a metabolic branching point occurs [Schmidt (2003)]. Currently the mean δ^{13}C of atmospheric CO_2 is ~-8‰. This value, however, changes seasonally with relative ^{13}C depletion during winter and enrichment during summer, particularly in the northern hemisphere [Levin et al. (1995)], and is becoming more negative over time (~ 0.02-0.03‰ year^{-1}, according to the data available from the CU-INSTAAR/NOAACMDLnetwork for atmospheric CO_2; http://*www.esrl.noaa.gov/gmd/*).

C3 plants fractionate during normal environmental conditions against the heavier $^{13}CO_2$ and prefer the lighter $^{12}CO_2$ during the photosynthetic uptake and incorporate thus leaving plant matter depleted in ^{13}C. Thereby, rates of carbon fixation and stomatal conductance are the primary factors determining carbon isotopic discrimination, which is thus directly related to the intrinsic water use efficiency (iWUE). The carbon isotope fractionation during diffusion through the stomata amounts to 4.4‰, and the discrimination during carboxylation of Rubisco (Ribulose-1,5-biphosphate carboxylase/oxygenase) to 27‰ [Farquhar et al. (1982), Farquhar et al. (1989)].
Water use efficiency (WUE) is defined as the ratio of A over E. Improved WUE allows greater biomass production per unit of rainfall [Condon et al. (2002), Condon et al. (2004)] thus offering a competitive advantage when water is limiting [Tsialtas et al. (2001)]. While iWUE is not directly linked to E, it shows a negative linear relation to c_i/c_a and therefore to $\Delta^{13}C$. The $\delta^{13}C$ of the plant biomass is a proxy for iWUE integrating over the time respective biomass was produced. iWUE of the plant can derived from gas exchange (A/g_s) and is then an instantaneous measure or from $\Delta^{13}C$. iWUE is used to describe tree water relations [Farquhar et al. (1989), Seibt et al. (2008)].
Different chemical compounds in plant leaves and other tissues may differ in their $\delta^{13}C$ and potentially also the bulk material of different tissues and organs might not be solely influenced by photosynthetic carbon isotope fractionation but also by post-photosynthetic processes. A detailed overview regarding the post carbon-fractionation and mixing processes within different plant organs is given by Gessler et al. (2014).
Also the $\delta^{18}O$ of plant material has been shown to contain essential information on water and carbon fluxes at the leaf, single tree and ecosystem scales. $\delta^{18}O$ in plant organic matter is mainly influenced by the $\delta^{18}O$ of the water taken up and by the evaporative enrichment of leaf water, where the primary assimilates are produced in. Spatial patterns in ^{18}O isotope ratios of precipitation were first observed in the 1950s by Dansgaard (1954). The author show that $\delta^{18}O$ in precipitation is well correlated with temperature. The value of this ^{18O} fractionation varies according to temperature (by an average change in $\delta^{18}O$ of 0.7‰ per °C) and altitude, resulting in a composition of precipitation that favours the heavier isotopes in positive relation with temperature, and in negative relation with altitude, distance from moisture sources and local precipitation amount [Dansgaard (1964)]. Therefore, analyses of precipitation $\delta^{18}O$ can reveal intra-annual to decadal information about regional weather patterns, and even large scale atmospheric circulation dynamics, depending on geographical region and season [Baldini et al. (2008), Field (2010)].
But $\delta^{18}O$ between surface water and ground water give no reliable values. Groundwater

generally represents a long term mean of δ^{18}O in precipitation and show no significant variations [Siegenthaler and Oeschger (1980), Saurer et al. (1997)]. Furthermore, no significant fractionation happens in general during the water uptake (source water) through the roots and within the transport to the leaves [White et al. (1985)]. Craig and Gordon (1965) and Dongmann et al. (1974) describe this effect and developed following equation:

$$\Delta_{\mathrm{e}} = \epsilon^{+} + \epsilon_{\mathrm{k}} + (\Delta^{18}\mathrm{O_v} - \epsilon_{\mathrm{k}})\frac{\mathrm{e_a}}{\mathrm{e_i}} \tag{1}$$

Where ϵ^{+} is the equilibrium fractionation between liquid water and water vapor $\sim 9.6‰$ at 20°C [Majoube (1971)], $\epsilon_k \sim 26.5‰$ is the kinetic fractionation as vapor diffuses from leaf intercellular spaces to the atmosphere [Farquhar et al. (1989)], $\Delta^{18}O_v$ is the isotopic enrichment of water vapor relative to the source water taken up by the plant and e_a/e_i is the ratio of ambient to intercellular vapor pressures. It is assumed that e_a/e_i is directly proportional with the relative humidity (rH) of the surrounding atmosphere, since rH is 100% within the intercellular air space. But the approach from Craig and Gordon (1965) and Dongmann et al. (1974) considered only the water composition at the site of evaporation, and not the whole leaf water.

The first fractionation of δ^{18}O in the leaf water starts during transpiration. The ^{18}O in leaf water enriched during the day, when the stomata are open for assimilation and transpiration (^{16}O evaporates easier than ^{18}O due to higher water vapor pressure). Consequently, the δ^{18}O of the leaf water contains a mixed isotopic composition of source water as well as the enrichment of ^{18}O during transpiration. Mean lamina mesophyll water is less enriched than the water at the evaporative sites, resulting in an isotopic gradient between the leaf vein and the evaporative sites. The steady-state isotopic enrichment of mean lamina mesophyll water can be explained by correcting Eqn 1 by the Peclet effect [Farquhar and Lloyd (1993)], as shown in Eqn 7. The Peclet effect is the net effect of the advection of unenriched source water to the leaf evaporative sites via the transpiration stream as opposed by the diffusion of evaporatively enriched water away from the sites of evaporation [Hommel et al. (2014)].

Another ^{18}O isotope signal fractionation happens from the leaf and stem water into organic matter transfer during carbon fixation. Two oxygen atoms are directly incorporated into organic matter from water, and a third one is derived from CO_2. In almost each metabolic step involves a certain degree of exchange of oxygen atoms between carbonyl groups and water [DeNiro and Epstein (1979), Farquhar et al. (1998)]. The oxygen equilibrium fractionation factor (ϵ_{wc}) resulting in carbonyl oxygen being approx. 27‰

more enriched than water [Sternberg (n.d.)]. These ϵ_{wc} finding is confirmed by several authors for different compounds: Yakir and DeNiro (1990) show it for cellulose, Barnard et al. (2007) found it for leaf soluble organic matter and Cernusak et al. (2003) as well as Geßler et al. (2007) found it in phloem sap sucrose.
Consequently, during drought it could be that the ^{18}O signal originates also partially from the stored carbohydrates e.g. starch remobilization 5 out of 11 oxygen atoms in sucrose generated from starch are exchanged with the surrounding non enriched water [Geßler et al. (2007)], so still 6 oxygen atoms and their $\delta^{18}O$ are retained from the storage compounds. This might cause the uncoupling in the isotopic signals between leaves and tree rings [Offermann et al. (2011), Pflug et al. (2015)]. According to Offermann et al. (2011) a greater contribution of xylem water $\delta^{18}O$ to the $\delta^{18}O$ of phloem organic matter in spring exists, than in summer and autumn. This is in agreement with the part of wood formation, in which the most assimilates are likely to incorporate from remobilized organic matter.[Helle and Schleser (2004)]. As a consequence of these processes, the initial oxygen isotope signal of the photosynthates might depending on species and environmental conditions be already altered when they arrive at the trunk cambium. Moreover, oxygen exchange occurs between sugars and reaction water during cellulose biosynthesis [Sternberg et al. (2006)]. A promising method to quantify ^{18}O in sugars, was currently developed by Lehmann et al. (2015). These authors show unrivalled precision for $\delta^{18}O$ analyses of various sugars by using a methylation derivatization.

Isotopic tracers

In order to track the fate of assimilated C (or water taken up by the roots), the natural isotopic signal is often not suited due to the various fractionation and isotope mixing proceses occuring. As a consequence isotopic tracer studies with a strong enrichment (or a depletion) of the rare stable isotope can be applied. Pulse-labelling of trees enables the quantification of newly assimilates produced, remobilization, half-live times of the C assimilated, C-partitioning and -sequestration from the leaf scale to the stand level. An extensive overview about tracing studies (fate and its release) in the ecosystem context is given by Epron et al. (2012). The review of Epron et al. (2012) lists 47 labelling studies in various ecosystem compartments. These authors have shown that new assimilates are rapidly transferred to belowground compartments, within few days or faster. Furthermore the authors concluded, that mean residence times (MRT) of labelled C in leaves and phloem sap are short, while those of sink organs (e.g. roots) are longer. The best estimate of the MRT of C is obtained by ^{13}C online measurements with

a high temporal resolution. To understand plant derived C input processes into the soil, it is important to trace seasonal C dynamics and allocation patterns. These patterns are responsible for the sink strength variations are most decisive drivers for biogeochemical C fluxes.

The combination of pulse labeling experiments with compound specific isotope analysis ensure an advanced approach to follow the ^{13}C label through the different NSC, which can be distinct in their temporal and spatial labelling [Rinne et al. (2012), Streit et al. (2013)].

About this study

How the changing climate is affecting C allocation within trees remains still unclear, because most studies have focused on growth responses to environmental changes and not on the transport pathways for C [Körner (2006)]. Because of that, the present study assess the interactions between the carbon balance and water relations in beech and co-occurring vegetation as affected by climate and climate-related environmental conditions (e.g. soil drought). This study spans this characterization from the leaf level to the area scale. The study combines (**chapter I**) natural abundance stable isotope analysis (δ^{13}C, δ^{18}O) and modeling the leaf water movement pathways, (**chapter II**) carbon allocation and transport patterns including analysis of different plant organs and (**chapter III**) a tree hydrodynamic model linked with measured stable isotope analyses in tree-ring cellulose.

Objectives and hypothesis

The objectives of this study is to characterize the interactions between the carbon balance and the water relations of beech and competing vegetation and the tree stand development of beech dominated forests as affected by climate and climate-related environmental conditions. This study attempt to connect plant physiological information with plant water and carbon balance by using stable isotopes, water and carbon flux analysis as well as modeling approaches. These modeling approaches reached from the leaf level up to the ecosystem scale.

Objective 1: Drought adaptation at the leaf level. Water use efficiency, mesophyll conductance and photosynthetic carbon isotope discrimination

Regulation of stomatal and mesophyll conductance are efficient mechanisms for optimizing the relationship between water loss and carbon uptake in plants when water availability is restricted. We assessed carbon isotope fractionation and derived water-use efficiency (WUE)-based drought adaptation strategies with respect to mesophyll conductance for different functional plant groups of the forest understorey. Moreover we aimed at assessing the mechanisms of and interactions between water and CO_2 conductance in the mesophyll.

Chapter I contains a greenhouse study assessing the drought response of g_m and interactions with leaf water movement (e.g. effective pathlength) among different forest species. We exposed *Impatiens noli tangere*, *Allium ursinum*, *Mercurialis annua*,*Acer platanoides*, *Fraxinus excelsior* and *Fagus sylvatica*, to two different soil water availability regimes (moderate drought vs. well watered control) and determine simultaneously gas

exchange and chlorophyll fluorescence in order to estimate g_m (according to Terashima and Ono (2002)). Chlorophyll fluorescence allows estimating electron transport rates to photosynthesis and photorespiration and to alternative electron acceptors. The relative proportions of photosynthesis and photorespiration depend on CO_2 and O_2 concentrations in the chloroplast and on the relative specifity factor of Rubisco (S_Γ). Photorespiration can be calculated from photosynthesis and electron transport and then used to compute c_c and thus g_m with the relative specifity of Rubiso. In addition, on the leaf level the present study focuses on the changes of g_m and its importance for WUE and iWUE strategies on the one hand and its effects on isotope fractionation models on the other hand. We compare $\delta^{13}C$ of newly assimilated sugars calculated from the simplified two step Farquhar model with measured values. The sensitivity of the Farquhar model [Farquhar et al. (1982)] for carbon isotope discrimination was tested to the observed variations in g_m in the different plant species during restricted soil moisture. In addition, we tested the influence of changes in g_m on current evaporative ^{18}O enrichment models by exploring (taking into account the Peclet number), if variations in g_m influence the relation between transpiration, the effective path length L, water isotopologue movement in the investigated plants.

We also assessed whether changes in g_m as a consequence of varied soil moisture affect evaporative enrichment models in a way according to Ferrio et al. (2009) and Ferrio et al. (2012).

Working hypothesis: An increased mesophyll conductance is decisive for the intrinsic water use efficiency (iWUE) optimization in plants, as it might differ between species and might be affected by soil water availability in different ways. Furthermore, according to Ferrio et al. (2012), we assume that the tortuosity for leaf water movement scales inversely with g_m in the different tree and herbaceous species indicating similar pathways and similar regulation for CO_2 and water in the mesophyll. Any drought-induced change in g_m would thus not only affect carbon assimilation directly but also leaf hydraulic properties.

Objective 2: Interaction between water and carbon balance at the single tree level. Carbon transport and metabolite fluxes during drought as well as intra- and inter-specific competition

Pulse-labelling of trees by using stable isotopes ensure to trace the fate of labelled CO_2 into the different plant organs (e.g. phloem, xylem, roots) and its release into other ecosystem compartments (e.g. soil, microbes, atmosphere). We studied C transport pathways, C allocation and velocity of newly assimilates during drought and competition at different harvest times. $^{13}CO_2$ pulse labeling experiments were conducted for

the relatively drought sensitive beech and a competitor (common maple). This experiment was carried out in a greenhouse with 3-year-old beech trees (*Fagus sylvatica*) and maple trees (*Acer platanoides*).The experimental treatments were: Competition treatments beech - monoculture; maple - monoculture; beech/maple - mixed culture and a moderate drought vs well watered control treatment. The short-term ^{13}C tracer movement (uptake, transport, allocation) was monitored by a full harvest approach. On the single tree level the experiment assessed the temporal courses of ^{13}C incorporation in non structural carbohydrates in the different plant organs. Moreover the the time course of the appearance of the label in soil respiration was assessed with high temporal resolution applying a cavity ring down isotopic laser spectrometer (Picarro).

Working hypothesis: A reduction in the water availability results not only in a reduction of assimilation but even more pronounced in a retardation and reduction of the carbon transport to heterotrophic tissues and from the plant to the soil. The response of reduced water availability and competition is more evident in mixed compared to the monoculture treatments, regarding C allocation to the roots. This change in the carbon allocation is on the long-term determined by changes in the source sink relation but other factors acting on short time scale, related to the physiology of phloem loading, transport and to leaf metabolic fluxes, are involved. Different species, especially drought-sensitive beech vs. competitor maple, differ in their water-related carbon allocation pattern and in the underlying mechanisms (e.g. iWUE, g_m, root respiration and root competition).

Objective 3: Water and carbon balance at the tree and stand level. Retrospective analysis of stable isotopes in tree rings, towards a mechanistic understanding of isotope fractionation and mixing processes from the leaves to the wood

Stable isotopes ($\delta^{18}O$ and $\delta^{13}C$) in tree rings are a promising tool in palaeoclimate research, provided attempts are made to disentangle climatic from local mixing processes [Gessler et al. (2014)]. However, various authors show clear responses in tree ring isotopes towards changing environmental conditions [Libby et al. (1976), Francey and Farquhar (1982)].

The $\delta^{18}O$ and $\delta^{13}C$ isotope signatures in tree rings of European beech (*Fagus sylvatica*) covering the last nine years were performed at an experimental submontane afforestation site (Tuttlingen, Germany), with long term baseline data on climate and soil parameters linked with a hydro-dynamic model approach (xylem water flow model, XWF). In order to assign the effects of local climate and intra-specific competition, the study in chapter III took the advantage of the two different aspects (NE vs. SW exposed) with significantly different local topo-climate treatments. Chapter III focuses on a tree hy-

drodynamic model based on tree architecture and sap flow data, linked with measured stable isotopes of extracted tree ring cellulose in mature beech trees. Isotope analysis was carried out for a 10-year chronology. The initial step on the way to extract retrospective physiological information from tree ring parameters ($\delta^{13}C$, $\delta^{18}O$) is the study of common signals within tree ring datasets (independently from physiological targets). Therefore, after trend analyzes of raw datasets, carefully adapted to every individual tree ring parameter, de-trending methods were applied to eliminate background and mixing influences. The isotope information was then translated into physiological information (e.g. iWUE). The additionally applied model approach follows the porous media approach using a dual permeability water flow and transport model originally developed for soils [Simunek et al. 2003]. The developed model describes a tree water flow and solute transfer in the xylem and phloem by including an additionally exchange term with wood. We developed and extended the simulation model for beech hydrodynamics in chapter III.

Working hypothesis: The water and carbon balance of beech is strongly affected by inter-annual climate variations and especially the extraordinary dry year 2003. Taking into consideration the isotope fractionation in the tree ring archive allows to identify the physiological responses of plants or trends retrospectively. Thereby, we hypothesized for the drought in 2003 growth will be reduced and water use efficiency will be affected. Moreover, we assumed that a mismatch between plant available water and evaporation demand occurred, leading to stomatal regulation and reduced water and C exchange at the leaf level. As a consequence, we expected that a shift in the A to g_s ratio towards increasing water use efficiency occurred. The first impact should be visible in the tree ring dimensions, the second should be reflected in the simulation of whole tree hydraulic conductance, and the third in an increased iWUE.

Chapter I

Drought response of mesophyll conductance in forest understory species - Impacts on water-use efficiency and interactions with leaf water movement

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Abstract

Regulation of stomatal (g_s) and mesophyll conductance (g_m) is an efficient means for optimizing the relationship between water loss and carbon uptake in plants. We assessed water-use efficiency (WUE)-based drought adaptation strategies with respect to mesophyll conductance of different functional plant groups of the forest understory. Moreover we aimed at assessing the mechanisms of and interactions between water and CO_2 conductance in the mesophyll. The facts that an increase in WUE was observed only in the two species that increased g_m in response to moderate drought, and that over all five species examined, changes in mesophyll conductance were significantly correlated with the drought-induced change in WUE, proves the importance of gm in optimizing resource use under water restriction. There was no clear correlation of mesophyll CO_2 conductance and the tortuosity of water movement in the leaf across the five species in the control and drought treatments. This points either to different main pathways for

CO_2 and water in the mesophyll either to different regulation of a common pathway.

Introduction

Currently climate models predict that many important forest regions in Central Europe will experience increased frequency and severity of drought periods [Ciais et al. (2005), IPCC (2007)]. Water restriction intensifies the general predicament of plant gas exchange, i.e. the loss of water to gain carbon [Chaves et al. (2003)] and thus the optimization between CO_2 uptake (A) and water loss (E) to increase water-use efficiency (WUE=A/E) is often an important adaptive advantage under drought conditions [Heschel et al. (2002), Aranda et al. (2012), Flexas et al. (2013)]. Improved WUE allows greater biomass production per unit of rainfall [Condon et al. (2002), Condon et al. (2004)] thus offering a competitive advantage when water is limiting [Tsialtas et al. (2001)].

Stomatal regulation in vascular plants is an efficient means for adjusting water use to changes in plant water supply and demand and this fine-tuned mechanism allows a rapid reaction to altered water availability, while attempting to maximize carbon uptake, thus optimizing the relationship between water loss and carbon uptake. At the leaf level the balance between CO_2 uptake and water loss is reasonably well understood [Farquhar and Richards (1984), Warren et al. (2001), Chaves et al. (2003)]. However, there is an increasing awareness that particular drivers of this balance such as g_m might vary more strongly than previously assumed [Warren and Adams (2006), Vrabl et al. (2009), Douthe et al. (2011), Douthe et al. (2012), Evans and Von Caemmerer (2013)].

Different species, provenances and cultivars differ in their ability to adapt stomatal conductance or leaf biochemical capacity for carbon fixation to optimize carbon gain with respect to water loss. Such differences in optimization strategies can strongly affect synecological interactions especially at sites exposed to periodic drought [Grams et al. (2007), Niinemets et al. (2009)]. Against this background, the importance of g_m to the relationship between E and A was brought back to general attention [Warren and Adams (2006), Aranda et al. (2007), Barbour et al. (2010)]. It is still under fierce debate how drought-related changes in g_m affect short- and longer-term WUE strategies of different species [Flexas et al. (2008), Flexas et al. (2013)]. Closing the stomata and thus reducing g_s in response to drought increases WUE but also decreases net photosynthesis. In theory the decrease in A could be compensated by means of increasing g_m with the result of increasing WUE not at the expense of A [Aranda et al. (2007)]. It is hypothesized that aquaporins, which facilitate the diffusion of CO_2 through cell membranes, are involved

in short-term changes in g_m [Flexas et al. (2006), Miyazawa et al. (2008), Uehlein and Kaldenhoff (2008), Evans et al. (2009)]. On the other hand, anatomical changes might be important for medium- to long-term acclimation to drought [Tosens et al. (2012)]. Changes in mesophyll conductance to CO_2 might also be coupled to changes in leaf hydraulic conductance [Ferrio et al. (2012)], which in turn can affect transpiration, mainly via stomatal regulation of leaf water potential [Sack and Holbrook (2006)]. In particular, several studies suggest that water movement through the mesophyll is not only apoplastic but can also be mediated by aquaporins via cell vacuoles (transcellular pathway) to the sites of evaporation [Steudle and Frensch (1996), Martre et al. (2002), Sack et al. (2004), Cochard et al. (2007), Kaldenhoff et al. (2008), Heinen et al. (2009), Pou et al. (2013)]. However, direct evidence of changes in mesophyll hydraulic conductance in response to drought is scarce, particularly due to the confounding effect of vein xylem embolism [Johnson et al. (2009)]. In this context, stable isotopes in leaf water offer an alternative approach to get insight into mesophyll limitations for water flow [Barbour and Farquhar (2004), Ferrio et al. (2009)].

Drought-induced changes in leaf hydraulic properties, e.g. a restriction of aquaporin-mediated transcellular pathways [Miyazawa et al. (2008), Pou et al. (2013)], are expected to increase the tortuosity for water movement through the mesophyll [Ferrio et al. (2009), Pou et al. (2013)], which in turn determines a key parameter in the models describing isotopic enrichment of leaf water, the effective pathlength of water movement from the xylem to the sites of evaporation (L) [Farquhar and Lloyd (1993), Barbour and Farquhar (2004)]. Taking advantage of this, Ferrio et al. (2012) showed that L, as a measure of tortuosity, was inversely related to leaf hydraulic conductance and g_m in grapevine. As a consequence they assumed that water and CO_2 could share, under certain circumstances, an important part of their respective diffusion pathways in the mesophyll, leading to coupled changes in water and CO_2 transport. In the same study, the tight inverse link between L and hydraulic conductance was further supported by a survey on literature data of 16 genera. Flexas et al. (2012) also reported a positive trend when comparing literature data on hydraulic conductance and g_m, but only in 20 out of the 28 genera studied.

Although in some cases g_m and hydraulic conductance have shown to be similarly restricted by aquaporin suppression [Miyazawa et al. (2008)], recent results indicate that the aquaporin-mediated regulation of diffusion can be different for CO_2 and H_2O [Otto et al. (2010), Kaldenhoff (2012)]. Briefly, this model proposes that aquaporin subunits with different affinities to CO_2 and H_2O compete in the formation of aquaporin complexes. In this regard, Flexas et al. (2012) showed evidence from tobacco mutants that

higher expression of a CO_2-aquaporin increased gm for CO_2 and at the same time increased L, thus breaking the correlation discussed above between g_m and L, and pointing to a negative relationship between mesophyll hydraulic conductance and g_m. Moreover, if the relative contributions of apoplastic vs symplastic pathways of water movement differ among species and/or environmental conditions we might expect more (relatively higher apoplastic contribution) or less (relatively higher symplastic contribution) common regulation of CO_2 and H_2O movement in the mesophyll.

Beyond the mechanisms controlling CO_2 and liquid water conductance, it is still an open question whether drought adaptation strategies involving g_m are of general importance. Whereas comparable declines in g_m and g_s in response to drought have been observed for several species [Flexas et al. (2009), Galle et al. (2009), Galle et al. (2011)], under certain conditions gm can be less sensitive than g_s [Duan et al. (2009), Galmés et al. (2011), Cano et al. (2013)], or even remain unaffected, despite strong reductions in g_s [Bunce (2009)]. On the other hand, we lack information about g_m-related strategies for drought adaptation of potentially competing or at least co-occurring species. Only recently it was observed that variations in g_m between functional groups can be small [Warren (2008)] in comparison with variations within these groups and even within cultivars of a particular species exposed to different environmental conditions [Flexas et al. (2008)]. In contrast, Peguero-Pina et al. (2012) showed that higher mesophyll conductance in the drought-adapted Abies pinsapo compared to the more mesic Abies alba resulted in higher A and WUE in the former species. In our study we assessed drought adaptation strategies with respect to mesophyll conductance of different functional plant groups of the forest understory. We exposed seedlings of the tree species *Acer platanoides* and *Fraxinus excelsior* as well as the herbaceous forest understory species *Impatiens noli tangere*, *Allium ursinum* and *Mercurialis annua* to moderate drought as normally expected in the forest understory, where extreme conditions (particularly humidity and temperature) are normally buffered by the overstory [Fotelli et al. (2003)], and monitored transpiration rate (E), assimilation rate (A), stomatal and mesophyll conductance, WUE and intrinsic water use efficiency (WUE_i=A/g_s).

Concerning the mechanisms of and interactions between water and CO_2 conductance in the mesophyll we took as our working hypothesis, based on the work of Ferrio et al. (2012), that the tortuosity for leaf water movement scales inversely with gm in the different tree and herbaceous species indicating similar pathways and similar regulation for CO_2 and water in the mesophyll. Any drought-induced change in g_m would thus not only affect carbon assimilation directly but also leaf hydraulic properties.

Materials and methods

Plant material and experimental set-up

Two different tree and three herbaceous species growing in the understory of mixed deciduous forests in Central Europe were selected for the experiments. *Fraxinus excelsior* (common ash) is known to be drought tolerant [Peltier and Marigo (1999)] and *A. platanoides* (Norway maple) was described as having a high WUE [Kloeppel and Abrams (1995)]. For the herbaceous species we have chosen two different life form types: *I. noli tangere* and *M. annua* as therophytes and *A. ursinum* as a geophyte. The tree species were obtained from a tree nursery (Handel, Metzingen, Germany). The 2-year-old *F. excelsior* seedlings were planted with soil container substrate (Kausek, Mittenwalde, Germany) in 5.5 l pots and the *A. platanoides* seedlings in 4 l pots. 9–12 g lime (Rüdersdorf, Germany) was applied per pot. The *I. noli tangere* and *M. annua* seeds were sourced from the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) and bulbs of *A. ursinum* were collected from a natural field site close to Müncheberg, Germany. The herbs grew in 15 l plant tubes per six individuals in the greenhouse. 45 g lime was applied for *I. noli tangere* and *Mercurialis annua*.
The plants were grown in a greenhouse at an average air temperature of 20°C. The photosynthetic photon flux density (PPFD) at the canopy level was kept at approximately 600 μmol $m^{-2}s^{-1}$ by applying supplemental illumination (bulb type NARVA NC 1000–00). The light period was adjusted to 16 h for the two tree species and to 14 h for the herbs. The bulk density of the mainly organic soil substrate was 0.12±0.01 g cm^{-3}. Volumetric soil water content (θ_s) was detected continuously with EC-5 soil moisture sensors (UMS Analytic systems, Munich, Germany) at a depth of 5–10 cm for herbs and at a depth 10–15 cm for the trees (Table 1). During the acclimation period in the greenhouse the pots containing the plants were watered every second day to field capacity.

The total sample size was 24 individuals for each tree species and 60 for each herb species. After 4–5 weeks of acclimation in the greenhouse half of the individuals of each species were exposed to soil water restriction for 10–21 days (see Table 1) whereas the other half (control) were well watered by keeping the soil moisture at field capacity.
Soil water potential (Ψ_s) was derived from θ_s according to Schindler et al. (2010). The thresholds of soil moisture tension for the forest species [Bittner et al. (2010)] were used and pots were carefully watered to reach these target values if (Ψ_s) fell below the threshold. The thresholds in soil moisture imply still a minimal root water uptake as defined in

Table 1: Characterization of the species and the experimental conditions. The plant age is given in years and for the two tree species the code for German provenance is given (811 07 and 800 04). Tree height before the treatment and number of individuals tested (N) in each treatment (drought and control) is also given. θ_s is the average of volumetric water content) during the drought in both treatments. The duration of the drought treatment is given in units of days (d).

Species parameter	*Fraxinus excelsior*	*Acer plata-noides*	*Mercurialis annua*	*Impatiens noli tangere*	*Allium ursinum*
Age (years)	2	2	1	1	5
Orignin	811 07	800 04	wild crops	wild crops	wild crops
Height (cm)	60-120	40-60	30-50	30-50	5-15
N	12	12	30	30	30
Duration (d)	21	14	17	17	10
Control					
θ_s (%)	48.1(±)4.1	44.4(±)1.4	46.7(±)1.3	45.9(±)3.5	49.4(±)1.9
Dry conditions					
θ_d (%)	20.8(±)2.7	18.9(±)1.7	24.3(±)0.4	11.3(±)0.2	33.8(±)1.0

Bittner et al. (2010); below the threshold value root water uptake is assumed to be zero. By doing so we exposed plants to moderate drought stress but avoided damage or death due to severe drought conditions. For the herbaceous species there are no threshold values given in the literature. Water supply was lowered until first clear signs of drought (wilting at noon) were visible.

Gas-exchange measurements and mesophyll conductance

A, E, g_s at different leaf intercellular CO2 concentrations (C_i) and different PPFD were recorded in all species, during the experiment using a GFS-3000 (Walz Measurement Instrumentation, Effeltrich, Germany). The measurements were carried out twice for each treatment, once before lowering soil moisture and once within the last three days before the end of the experiment. Simultaneously electron transport rate (ETR) of the leaves was estimated as follows for each CO_2 concentration (Eqn 2)

$$\mathrm{ETR} = \frac{\Delta \mathrm{F}}{\mathrm{F_m}'} \cdot 0.84 \cdot \frac{\mathrm{PPFD}}{2} \tag{2}$$

where the effective quantum yield of PSII ($\Delta F/F_m'$) was calculated as (F_m'-F)/F_m'. F is the fluorescence yield of the light-adapted sample and F_m' is the maximum fluorescence obtained when a saturating light pulse is superimposed on the prevailing environmental light levels [Schreiber and Bilger (1993)]. The intensity and duration of the saturating light pulse was adjusted according to the fluorescence kinetics (http://*www.walz.com /downloads/manuals/gfs − 3000/gfs − 3000_Manual_PanelPC.pdf*). The pulse duration was 800 ms, and the pulse intensity was set to maximum, which equals <4500 μmol $m^{-2}s^{-1}$ (http://*www.walz.com/products/gas_exchange/gfs − 3000/spec_led_array.html*). We acknowledge that recent results from Loriaux et al. (2013) have shown that a single multiphase flash of sub-saturating intensity might further improve the accuracy of fluorescence measurements. This procedure was not possible with the PAM fluorometer of the GFS-3000, which however has the advantage compared to other commercially available systems of integrating both gas exchange and fluorescence on an 8 cm^2 area. The factor of 0.84 represents the total light absorption of the leaf. This empirical factor takes into account that only a fraction of the incident light is absorbed [Rascher et al. (2000)]. Under stress, leaf absorbance might, however, decrease or increase – depending on the species – and might reach values below 0.7 [Delfine et al. (1999)] or above 0.9 [Loriaux et al. (2013)]. In order to take such potential variation of the light absorption factor into account we included variations of ETR in a sensitivity analysis for g_m (see below).

For the calculation of CO_2 concentration at the sites of carbon fixation (C_c) according to Eqn 3 the ribulose 1.5-bisphosphate carboxylase/oxygenase (RubisCO) specificity factor (S_Γ)was estimated. To derive this parameter we computed Γ^*, the CO_2 compensation point in the absence of day respiration for all plants and treatments. In other words it is the CO_2 concentration where CO_2 uptake by carboxylation is matched by photorespiratory CO_2 release, and where the rate of CO_2 release is day respiration [Laisk (1977), Atkin et al. (2000)]. Γ^* was calculated from A/C_i curves [Farquhar et al. (1980), Brooks and Farquhar (1985), Long and Bernacchi (2003)] separately for plants from the drought treatment and the controls and applied to quantify the S_Γ. Five A/C_i curves were measured at different light intensities (50, 100, 200, 400 and 700 μmol $m^{-2}s^{-1}$) with eight different CO_2 concentrations per curve, ranging from 25 to 400 ppm. We also compared the initial slopes of the A/C_i curves between drought treatment and control since changes of this slope are assumed to be a symptom of stomatal patchiness [Grassi and Magnani (2005)]. The slopes did not vary between treatments with the exception of *I. noli tangere* and *A. platanoides*, where slopes slightly diverged but only at the lowest

light intensity, pointing to the fact that calculation of C_i was not affected by patchy stomatal closure for the vast majority of the conditions applied.
The calculation of C_c was carried out as follows:

$$C_c = \frac{O}{S_\Gamma} \cdot \frac{ETR + 8 \cdot (A + R_l)}{2ETR - 8 \cdot (A + R_l)} \quad (3)$$

We assumed O the O_2 concentration in the chloroplast to be 0.21 molmol^{-}1. Rl is the mitochondrial respiration rate in the light on a leaf area basis. g_m was calculated (Eqn 4) as follows according to Terashima and Ono (2002).

$$g_m = \frac{m_i \cdot m_c}{m_c - m_i} \quad (4)$$

Where m_i is the slope of A/C_i curves and m_c is the slope of A/C_c curves at a defined light intensity at low absolute CO_2 concentrations. m_c and m_i were calculated from the linear regression between A and the corresponding C_c and C_i values below 150 μmol mol^{-1}, respectively. The method of Terashima and Ono (2002), which is a modified version of the approach described by Epron et al. (1995) and where C_i is varied in a range with RubisCO limitation, compares well with other variable or constant J methods commonly applied to determine g_m [Pons et al. (2009)].
Diel course and water extraction After the A/C_i curves, diel courses of gas exchange combined with plant tissue sampling for isotope analyses were carried out for each species. Measurements for A, E, g_s, PPFD, leaf temperature, specific leaf area, leaf length, ambient temperature and relative humidity were carried out at 03:00 h in the dark and during the light period at 09:00 h, 15:00 h and at 21:00 h. At the same time points five leaves per plant and stem wood (trees) or root crowns (herbs) of three individuals per species were harvested.
Two leaves were used to analyze the carbon isotopic composition ($\delta^{13}C$) of leaf water-soluble organic matter (WSOM). With one leaf we estimated leaf water content (WC). Extraction of WSOM and determination of WC have been described in detail by Barnard et al. (2007). The last two leaves were placed together in glass tubes (Schott-Duran, Mainz, Germany), immediately frozen in liquid nitrogen and stored at -30°C. These leaves and the stem wood (with the bark removed) and the root crown (hypocotyl) were used for tissue water extraction by cryogenic vacuum distillation (Ferrio et al. (2009)). For *A. ursinum* only the middle part of the long monocotyledon leaf was sampled and also gas exchange as performed with this part of the leaf.

Isotopic measurements and calculations

δ^{18}O in water extracted from leaves, root crowns and stem wood was determined using a TC/EA (high temperature conversion/elemental analyzer: ThermoFinnigan, Bremen, Germany) coupled with a DeltaPlus XP mass spectrometer via a ConFlo III interface. The precision of the measurement was <0.15‰ Values are given in delta (δ) notation (in ‰) relative to the standard VSMOW (Vienna Standard Mean Ocean Water).
The extracted water of the root crown or the stem wood was considered as source water and its δ^{18}O value was applied to calculate the observed evaporative enrichment of leaf water $\Delta^{18}O_L$.

$$\Delta^{18}\mathrm{O_L} = \frac{\delta^{18}\mathrm{O_L} - \delta^{18}\mathrm{O_{source}}}{1 + \delta^{18}\mathrm{O_{source}}} \tag{5}$$

Where $\delta^{18}O_L$ is the oxygen isotopic composition of leaf water and $\delta^{18}O_{source}$ is the isotopic composition of the source water.
Leaf water enrichment during the diel courses was also modeled as described in detail by Barnard et al. (2007) to obtain the scaled effective pathlength L. First, steady-state isotopic enrichment of ^{18}O over source water at the site of evaporation in the leaf (Δ_e) under steady state conditions was calculated as follows (Craig and Gordon (1965), Dongmann et al. (1974)):

$$\Delta_\mathrm{e} = \epsilon^+ + \epsilon_\mathrm{k} + (\Delta^{18}\mathrm{O_v} - \epsilon_\mathrm{k})\frac{\mathrm{e_a}}{\mathrm{e_i}} \tag{6}$$

Where ϵ^+ is the equilibrium fractionation between liquid water and water vapor: ϵ_k is the kinetic fractionation as vapor diffuses from leaf intercellular spaces to the atmosphere [Farquhar et al. (1989)], $\Delta^{18}O_v$ is the isotopic enrichment of water vapor relative to the source water taken up by the plant and ea/ei is the ratio of ambient to intercellular vapor pressures. At each time point during the diel course the δ^{18}O of the atmospheric water vapor in the greenhouse was analyzed by using a Picarro Isotopic Water Analyzer L2120-i (Sunnyvale, CA).
Average lamina mesophyll water is less enriched than the water at the evaporative sites, resulting in an isotopic gradient between the leaf vein and the evaporative sites. The steady-state isotopic enrichment of mean lamina mesophyll water (Δ_{LsP}) can be de-

scribed by correcting Eqn 6 for the Peclet effect [Farquhar and Lloyd (1993)], as shown in Eqn 7. The Peclet effect is the net effect of the advection of unenriched source water to the leaf evaporative sites via the transpiration stream as opposed by the diffusion of evaporatively enriched water away from the sites of evaporation.

$$\Delta_{\mathrm{LsP}} = \frac{\Delta_{\mathrm{e}}(1 - \mathrm{e}^{-\wp})}{\wp} \quad \text{with} \quad \wp = \frac{\mathrm{E} \cdot \mathrm{L}}{\mathrm{C} \cdot \mathrm{D}} \tag{7}$$

$\wp$ is the Peclet number, E the leaf transpiration rate (mol $m^{-2}s^{-1}$), L is the scaled effective path length (m) for water movement from the xylem to the site of evaporation, C the molar concentration of water (mol ml^{-3}), and D the diffusivity (m^2s^{-1}) of the $H_2{}^{18}O$ isotopologue in normal water. The scaled effective path length as a measure for tortuosity was estimated by fitting the non-steady state model to the observed $\Delta^{18}O_L$ at 15:00 h under expected steady state conditions that typically occur in the afternoon. Under non steady-state conditions, the enrichment of mean lamina mesophyll water above source water (Δ_{LnP}) was calculated following Farquhar and Cernusak (2005):

$$\Delta_{\mathrm{LnP}} = \Delta_{\mathrm{LsP}} - \alpha^{+}\alpha_{\mathrm{K}}\left(\frac{1 - \mathrm{e}^{-\wp}}{\wp}\right)\left(\frac{\mathrm{d}(\mathrm{W}\Delta_{\mathrm{LnP}})}{\frac{\mathrm{dt}}{\mathrm{gw_i}}}\right) \tag{8}$$

where $\alpha^+ = 1 + \epsilon^+$ and $\alpha_k = 1 + \epsilon_k$, W is the lamina leaf water concentration (mol m^{-2}), t is time (s), g is the total conductance to water vapor of stomata and boundary layer (mol $m^{-2}s^{-1}$), and w_i is the mole fraction of water vapor in the leaf intercellular air spaces (mol mol^{-2}). An iterative solution was calculated with the Solver function in Excel. The model requires initial values for Δ_{LnP} and W for a time point (t_0 –1) preceding the first observation. To initialize the model we took the values from the last measurement.

In WSOM extracts, $\delta^{13}C$ was determined by combusting the samples in a Flash HT elemental analyzer (ThermoFinnigan) coupled via a ConFlo III interface to a Delta V advantage isotope ratio mass spectrometer. The precision of the measurement was <0.10‰. Small delta values are given relative to the standard VPDB.

We determined $\delta^{13}C$ of atmospheric CO_2 ($\delta^{13}C_{CO_2}$) and CO_2 concentration (c_a) during the diel courses with a G2101-i Picarro Isotopic CO_2 Analyzer to calculate photosynthetic carbon isotope discrimination $\Delta^{13}C$.

$$\Delta^{13}\mathrm{C} = \frac{\delta^{13}\mathrm{C}_{\mathrm{CO_2}} - \delta^{13}\mathrm{C}_{\mathrm{L}}}{1 + \delta^{13}\mathrm{C}_{\mathrm{L}}} \tag{9}$$

where $\delta^{13}C_L$ is the carbon isotopic composition of the leaf WSOM. WUE_i describes the ratio between A and g_s and was calculated from $\Delta^{13}C$ according to Eqn 10 [Farquhar et al. (1982), Seibt et al. (2008)].

$$WUE_i = \frac{C_a}{1.6}\left(\frac{b - \Delta^{13}C}{b - a}\right) \quad (10)$$

Where a is the carbon isotope fractionation during diffusion through the stomata. b is normally defined as the discrimination during carboxylation of RubisCO which amounts to 30.5‰ in the relevant (gas) phase (i.e. to 29.5‰ in the liquid phase; Tcherkez et al. (2013)). We, however, took a value that takes into account the typical drop in pCO_2 from the intercellular spaces to the sites of carboxylation, and b=26‰ as we used it is a reasonable such number empirically. We averaged the WUE_i values of the leaves sampled at 09:00 h, 15:00 h and at 21:00 h (i.e. during the light period).

Statistical analyses

All measured variables (under dry conditions and in control treatment) were first characterized by descriptive statistics (means and standard deviations of the means). The error bars in the figures represent the standard deviation (SD). Statistical analyses and fittings were carried out with R 2.8.0 (R Development Core Team 2010) with the nlme package for linear mixed effect models [Pinheiro et al. (2008)] and SIGMA-PLOT 12.0. Exponential curves were fitted by using SIGMA-PLOT 12.0. Student's t-test and Wilcox rank test were utilized to determine significance of difference between the two treatments.

Results

Gas-exchange and mesophyll conductance

The light-response curves of A, E and gs at 400 ppm CO_2 for the five species under control and reduced water supply conditions are given in Fig. 2, the maximum values (A_{max}, E_{max} and $g_{s\,max}$) calculated from the lightresponse curves are shown in Fig. 2. The water restriction treatment for *I. noli tangere* caused a clear decrease in A (Fig. 2a), E (Fig. 2b) and g_s (Fig. 2c) at all light levels measured as well as a significant decrease in the calculated maximum values A_{max}, E_{max} and $g_{s\,max}$(Fig. 3).

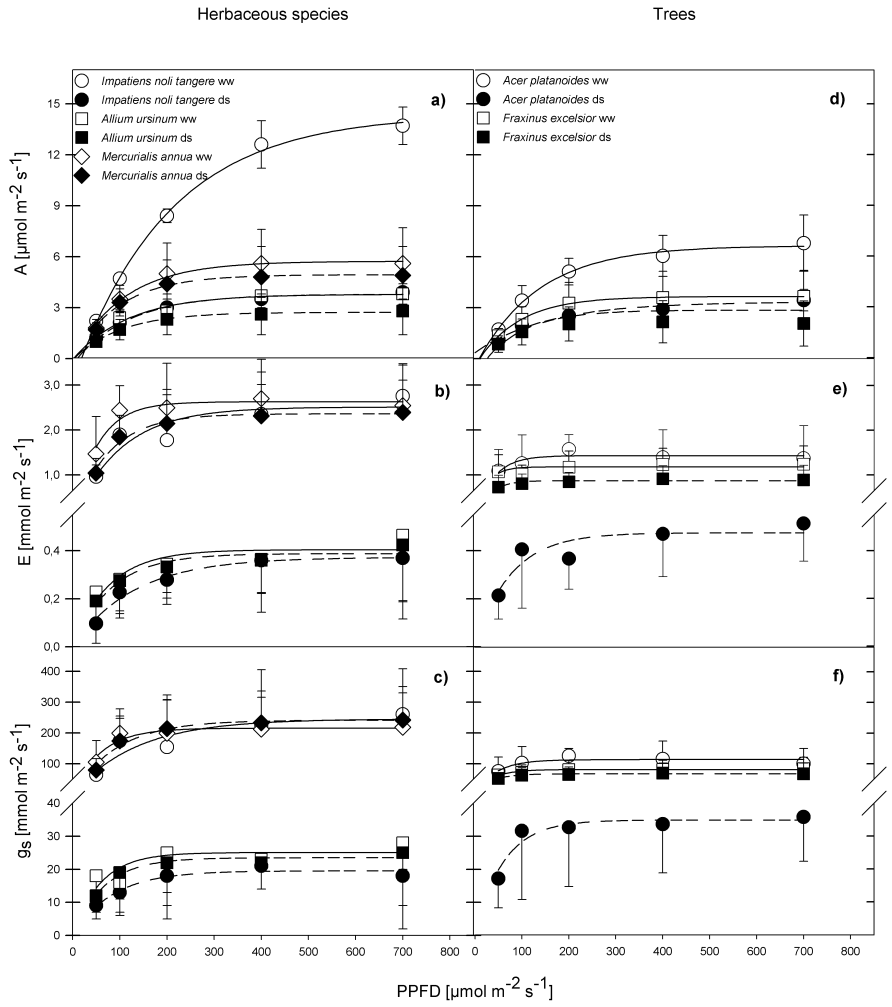

Figure 2: Light response of photosynthesis (A), transpiration (E) and stomatal conductance for H_2O (g_s) in control and drought treatments. In (a)-(c) the herbaceous species (n=4–5) and in (d)–(f) the trees (n=10) are displayed. The regression lines in (a) and (d) are based on Schulte et al. (2003), the regression lines in (b), (c), (e) and (f) are based on exponential fitting. Open symbols: well watered controls (ww), black symbols: dry conditions (ds), solid line: regressions line of well watered, dotted line: regressions line of dry conditions. Data shown are means(±)standard deviation. All measurements were performed at 400 ppm CO_2 with a leaf temperature of 24(±)1°C and at 60(±)3% relative humidity.

In contrast, we did not observe clear drought effects on E and gs for A. ursinum (Fig. 2b, c) but A_{max} was slightly lower than the control under the drought treatment (Fig. 3). No significant effect of drought on A, A_{max}, E, E_{max}, g_s and $g_{s\ max}$ were recorded

for M. annua (Figs 2a–c and 3). The two tree species clearly differed in their reaction to the drought treatment. Whereas only the light-response of A was slightly affected by drought in F. excelsior causing a significant reduction of A_{max} (Fig. 3), A, E and g_s decreased strongly in A. platanoides at all light intensities measured (Fig 2d–f). Consequently, the maximum values A_{max}, E_{max} and $g_{s\ max}$ were also clearly and significantly reduced by the drought treatment for A. platanoides (Fig. 3).

Since g_m is known to scale more [Evans and Von Caemmerer (1996)] or less (Warren and Adams (2006)] clearly with A, we plotted A vs g_m for the light-response experiment. In general we observed a linear relationship between A and g_m for a given species (Fig. S1A–E) but in two species (*I. noli tangere* and *A. platanoides*) the slopes of the regression lines were lower in the drought treatment than in the control. In *A. ursinum* and *M. annua* the A vs g_m relationship was very similar between the treatment and the control and for *F. excelsior* a clear regression could not be obtained for the drought conditions due to the low assimilation rates. Especially at lower g_s, and A under drought, the accuracy and precision of the measurements and estimates of the input parameters as given in the Eqns 2–4 might strongly influence the calculation of g_m. To assess this influence we performed sensitivity analyses and show as examples the results of a deviation in A, c_i and ETR as well as a variation in the estimate of Γ^* on g_m at A_{max} for *I. noli tangere* in the drought treatment and the control (Fig. S2). The effect of %-deviations of the input parameters were more pronounced in the drought treatment and the strongest effect was observed for deviations of A where a variation of (±)5% caused a change in g_m of ca. 60 mmol $m^{-2}s^{-1}$ (49%). g_m was less sensitive to deviations of ETR (which also includes deviations from the estimated leaf absorbance) and a variation of (±)5% caused g_m to change by 39 mmol $m^{-2}s^{-1}$ (i.e. 31%).

The relationship between stomatal and mesophyll conductance for the different species under drought and control conditions (with the variation of both parameters driven by varying light intensity) is shown in Fig. S1F–J. In *A. ursinum*, *M. annua* and – at least hinted at – in *F. excelsior*, g_s and g_m were regulated more or less concertedly independent of the drought or control treatment. For *I. noli tangere* reduced water supply led only to a slight increase of g_s with light intensity as shown in Fig. S1F, but in contrast, g_m increased strongly reaching values up to 135 mmol $^{-2}s^{-1}$. In *A. platanoides* g_m showed a comparable range along the PPFD gradient in the drought and in the control treatment but at very different g_s ranges (control: >100 mmol $m^{-2}s^{-1}$: drought between 15 and 30 mmol $m^{-2}s^{-1}$).

g_m at A_{max} ($g_{m\ max}$) was affected by the drought treatment in some species. It significantly decreased by approximately 50‰ under dry conditions for *A. ursinum* (Fig.

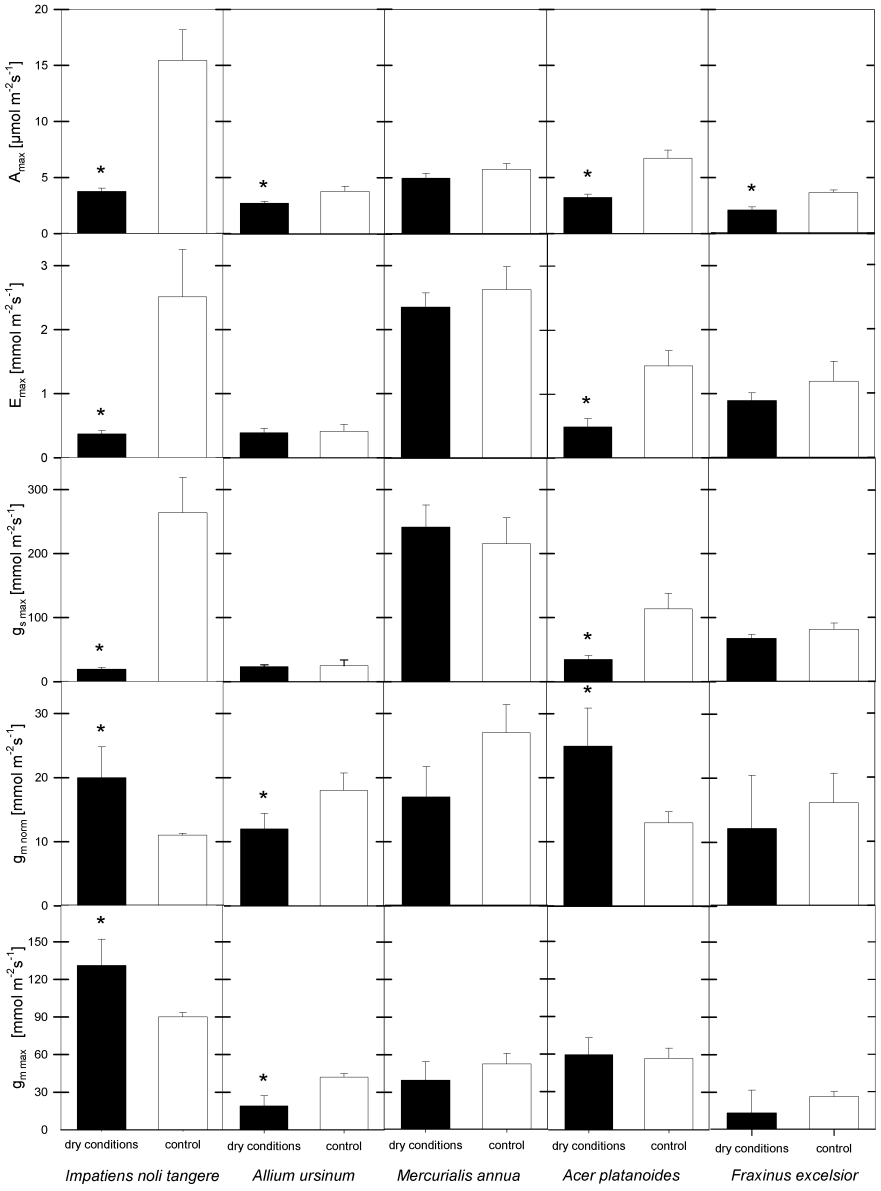

Figure 3: Cardinal points of the light response curves in the drought and control treatments. The figure shows the calculated A_{max} (μmol $m^{-2}s^{-1}$), E_{max} (mmol $m^{-2}s^{-1}$), $g_{s\ max}$(mmol $m^{-2}s^{-1}$) from the light response curves in Fig. 2. In addition g_m calculated for an A of 2 μmol $m^{-2}s^{-1}$ ($g_{m\ norm}$), mmol $m^{-2}s^{-1}$) and at A_{max} ($g_{m\ max}$, mmol $m^{-2}s^{-1}$) are shown. Significant differences ($P<0.05$) are marked with *.

3). In *M. annua* and *F. excelsior* a slight albeit insignificant decrease of $g_{m\ max}$ upon drought was observed, whereas in *I. noli tangere* $g_{m\ max}$ increased under drought by 45‰. In *A. platanoides* $g_{m\ max}$ was not clearly influenced by the treatment. Since g_m scales with A and A_{max} was significantly reduced upon drought in this species we additionally calculated a normalized $g_{m\ norm}$ for a given A of 2 μmol $m^{-2}s^{-1}$ (from the linear relationship between A and g_m as shown in Fig. S1) in Fig. 3. It becomes obvious here that for a given A, reduced water supply caused a significant increase in g_m for *A. platanoides* and *I. noli tangere*.

Oxygen isotope enrichment and tortuosity of water movement in the leaf

Measured leaf water $\Delta^{18}O_L$ showed maximum values during the light period in all species (Fig. 4) with more or less clear diel courses of leaf water enrichment. There as no clear difference in the absolute values and in the diel patterns between the drought and control treatments. Predicted Δ_e clearly overestimated measured $\Delta^{18}O_L$ in all species in both treatments with the exception of *A. platanoides*. In the other four species the consideration of the Peclet effect (Δ_{LsP}) greatly improved the predictions during the light period. Taking into account the isotopic non-steady state of leaf water (Δ_{LnP}) improved the predictions especially in the night and directly before dusk but only slightly improved the estimates during the rest of the light period. The effective pathlength L obtained from fitting the steady state (Δ_{LsP}) model to $\Delta^{18}O_L$ at 15:00 significantly increased under reduced water supply in *I. noli tangere*, *A. ursinum* and *A. platanoides* and stayed constant in *M. annua* and *F. excelsior* (Fig. 5).
When plotted against E for all species and treatments, it is visible that L increases with decreasing E below a threshold of approximately 0.5 mmol $m^{-2}s^{-1}$ (Fig. 6). At higher transpiration rates this relationship was not present. The exponential fit displayed in Fig. 6 resulted in an R^2 of 0.98. In contrast, there was no clear relationship between $g_{m\ max}$ and L.

Water-use efficiency

Gas-exchange derived WUE (as calculated from A_{max}/E_{max}) and WUE_i ($A_{max}/g_{s\ max}$) showed comparable patterns among species. In *I. noli tangere* and *A. platanoides* both parameters tended to increase under reduced water supply, whereas a tendency for a decrease was observed in *A. ursinum* and no change in the other two species (Fig. 5).

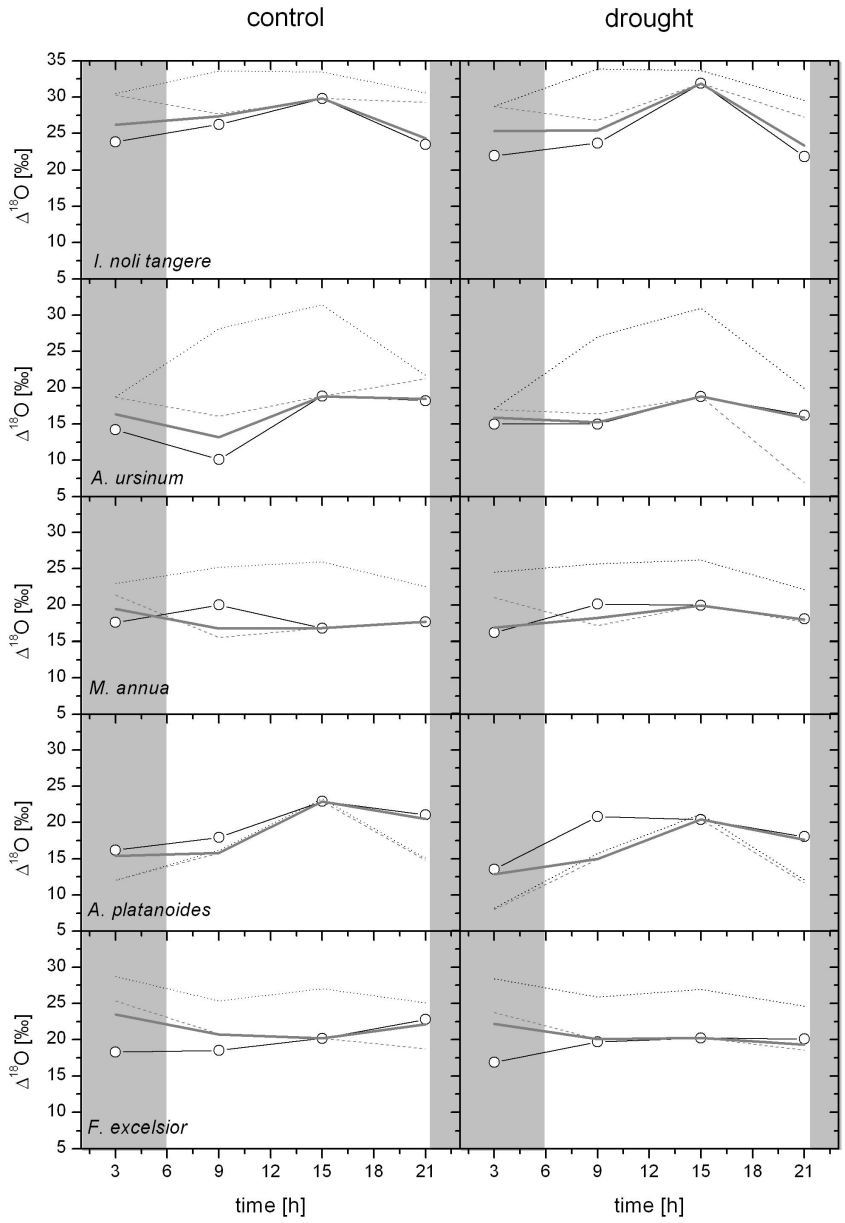

Figure 4: Diel course of observed mean lamina leaf water enrichment ($\Delta^{18}O_L$) and enrichment predicted from models of different complexity. $\Delta^{18}O_L$ is denoted by the white circles connected by black lines. Enrichment at the site of evaporation (Δ_e) is shown as black dotted line, modeled leaf water enrichment under steady state (Δ_{LsP}) by a gray dashed line and under non-steady state (Δ_{LnP}) by a gray bold line. Gray shadowed areas denote the dark periods.

WUE_i derived from $\delta^{13}C$ of WSOM corroborate the findings from the gas exchange measurements and indicates that the short-term integrating values (gas exchange measurements) are representative for the longer term ($\delta^{13}C$ of leaf WSOM). A correlation analysis between the change in WUE_{max} as a consequence of soil water restriction on the one hand and the related change in L, $g_{s\ max}$) or $g_{m\ max}$ on the other hand for all five species, produced significant results only for $g_{m\ max}$ (R^2 =0.77 P =0.04). The positive slope of the regression line in Fig. 7 indicates that across all species tested an increase in mesophyll conductance goes along with an increase in WUE.

Discussion

The ability to increase WUE is a competitive advantage for plants under water limiting conditions [Richards et al. (2002)]. Reduced soil water availability resulting from the drought treatment had effects both on A and E in some of the species examined in this study. In all species with the exception of M. annua maximum photosynthesis rate decreased as a consequence of drought but only in *I. noli tangere* and *A. platanoides* were E_{max} and $g_{s\ max}$ decreased significantly during the water restriction regimes applied. Generally, it is assumed that stomatal closure is the first response to drought. However, Flexas and Medrano (2002) suggested that ribulose bisphosphate regeneration and ATP synthesis and thus photosynthesis rate might be impaired as an early response to water restriction when g_s is still high. Such a response of A matches our observations.
The aim of our experiment was to apply a moderate drought treatment without irreversibly damaging plants and plant function and in a range realistic for the plants in their natural habitat. Climate models project higher variability of rainfall in future [IPCC (2007)], but refer Sun et al. (2012) causing periods with higher rainfall alternating with drier periods. Even though intensive summer droughts similar to the drought in 2003 [Bréda et al. (2006)] might occur more frequently in the future, mainly moderate droughts are to be expected for forest understory species as the herbs and tree seedlings examined here. This is because the forest understory experiences damped environmental fluctuations, due to shelter by the overstory tree canopy [Fotelli et al. (2003)]. Temperatures at the forest floor show lower amplitude compared to open vegetation and humidity levels are higher, reducing evapotranspiration and thus maintaining relatively high soil moisture compared to grassland or arable fields [Prescott (2002)]. These rather mild drought conditions obviously caused impairment in the functioning of photosynthesis in *A. ursinum* and *F. excelsior* before any stomatal response could be observed. As a consequence of the different A and E responses, WUE increased upon drought in *I. noli*

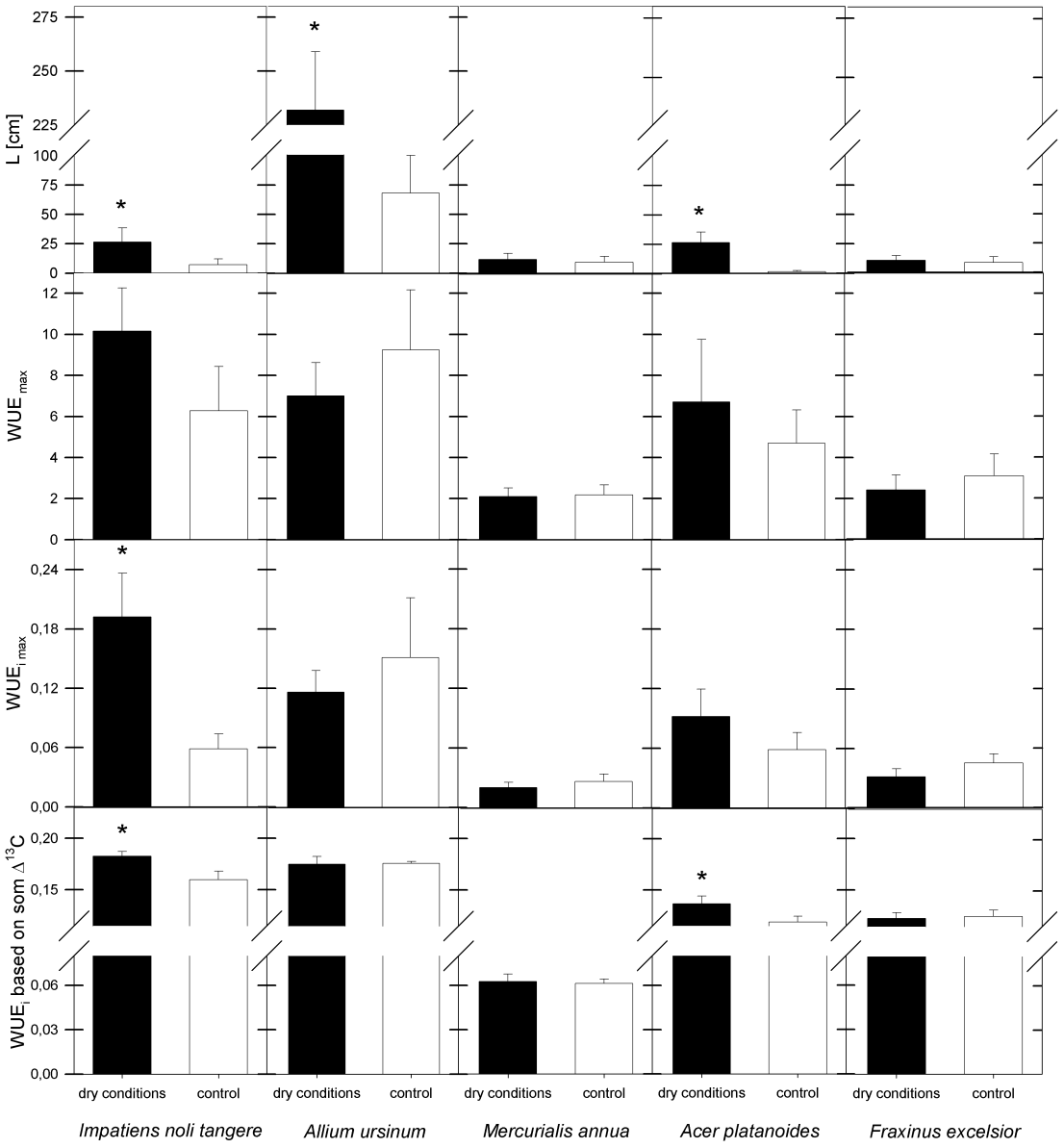

Figure 5: Calculated effective pathlength for water movement in the leaf L (cm), maximum water-use efficiency WUE_{max} (μmol mmol^{-1}) and $WUE_{i\ max}$ (μmol mmol^{-1}), as derived from the light curves and intrinsic WUE derived from the Δ^{13}C of leaf water soluble organic matter WUE_i(μmol mmol^{-1}) as determined as daytime averages during the diel courses. Error bars denote standard deviation. Significant differences (P<0.05) are marked with *

tangere and *A. platanoides* whereas it stayed constant in *M. annua* and even decreased in *A. ursinum* and *F. excelsior*.
In two species we observed a clear drought effect on the A vs g_m relationship: for both *I. noli tangere* and *A. platanoides* g_m increased more strongly with A in the drought treatment as compared to the control. Moreover, in these two species the coordinated adjustment of g_m and g_s differed between the two treatments. As a consequence of these altered relationships, $g_{m\ max}$ (i.e. g_m at light saturation and thus maximum A) increased (*I. noli tangere*) or remained constant (*A. platanoides*) in the drought treatment even though A_max and $g_{s\ max}$ decreased. In these two species WUE (WUE_max in Fig. 5) also increased upon drought, whereas it either remained constant or even decreased for the other species tested. The fact that an increase in WUE was observed only in the two species that increased g_m (either g_max or at least $g_{m\ norm}$ at a given A) in response to drought and that the change in $g_{m\ max}$ (but not in $g_{s\ max}$) was significantly correlated with the change in WUE due to the drought treatment (Fig. 7) proves the importance of g_m in optimizing resource use under water restriction. Indeed, a recovery in g_m (but not g_s) after prolonged drought conditions has been reported for some species [Galle et al. (2009), Galle et al. (2011)], as a mechanism to restore assimilation rates after long water restriction.

When assessing mesophyll conductance, especially under conditions where gs and A are rather low such as in drought experiments errors in the measurement and estimates of the input parameters can significantly affect the calculation of g_m [Pons et al. (2009)]. Our sensitivity analysis showed that deviations in single input parameters from -5% to +5% of the measured mean values can cause changes in g_m of up to 49% (for A), which is in agreement with the studies of Pons et al. (2009). This sensitivity of g_m needs to be taken into account when interpreting our data (and g_m data in general) and we need to be cautious not to overemphasize small differences as a result of a treatment. We, however, consider the difference in the relationship of A vs g_m between the two treatments for *I. noli tangere* and *A. platanoides* clearly large enough to be taken as a drought effect. The strong sensitivity of g_m to variations in A calls for the use of gas exchange cuvettes as large as possible [Pons et al. (2009)] and thus justifies the application of our system, which provided a chamber area of eight cm^2. In addition, stomatal patchiness might invalidate calculation of C_i [Grassi and Magnani (2005)] and thus g_m. Our analysis of A/C_i curves showed no symptoms of stomatal patchiness for almost all conditions, which is in agreement with findings that mainly a rapid strong dehydration causes patchy stomatal closure [Gunasekera and Berkowitz (1992), Kubiske and Abrams

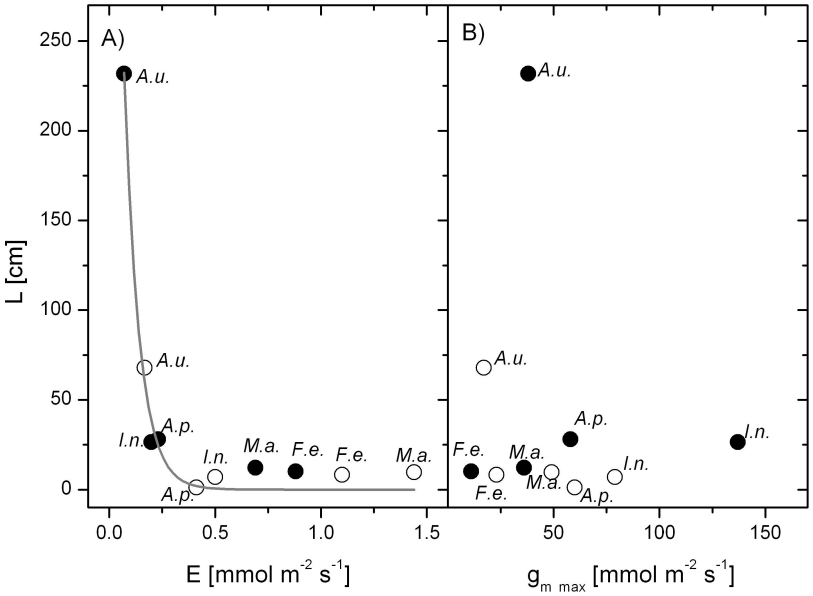

Figure 6: Relationship between the scaled effective pathlength L and (A) transpiration E at 15:00 h and (B) $g_{m\ max}$. Data shown are mean values for species in a given treatment (n=3). White symbols denote the control, black symbols the drought treatment. In (A) a first order exponential decay function (gray line) has been fitted to the data ($R^2 = 0.98$). A.u. (*Allium ursinum*), A.p. (*Acer platanoides*), I.n. (*Impatiens noli tangere*), M.a. (*Mercurialis annua*), F.e. (*Fraxinus excelsior*).

(1994)].

It might be additionally assumed that under low light only the mesophyll surface in the top part of the leaf is taken into account for mesophyll conductance since under these conditions photosynthesis mainly occurs in that part of the leaf and thus g_m values cannot be compared with those under high light conditions. We, however argue that such a pattern occurs under all light intensities. In leaves that receive light from above, the chloroplasts at the bottom of the leaf are 'shade' chloroplasts (compared to those at the top of the leaf), so that even in high light applied to the upper surface these bottom chloroplasts still only do a low portion of the leaf's photosynthesis. Indeed it has been shown [Farquhar (1989)] that the optimal performance of such a leaf will be to have the photosynthetic capacity (Rubisco activity and electron transport activity per unit leaf area) vary through the leaf in the same proportion as the light absorbed. This is regardless of the amount of chlorophyll, which determines the absorptance of the leaf

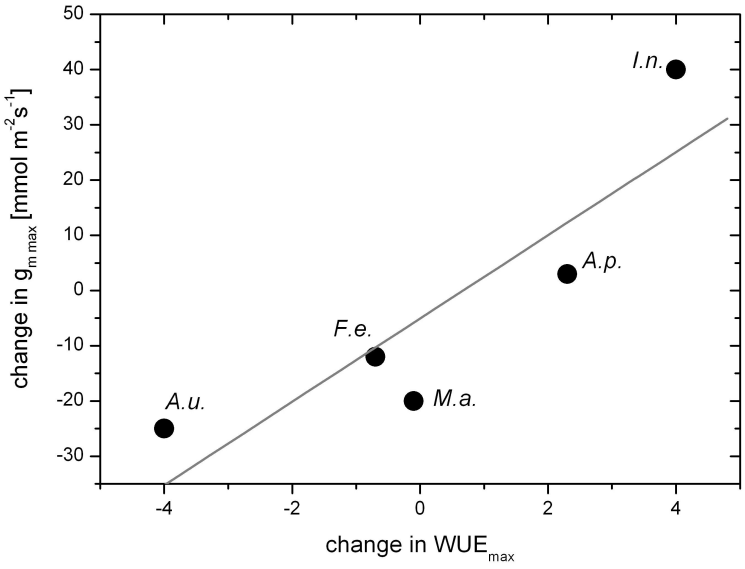

Figure 7: Change in $g_{m\ max}$ as a response to the drought treatment plotted against the change in water-use efficiency (WUE_max) for the five species examined. The regression line is significant at P = 0.04 (R^2 = 0.77). A.u. (*Allium ursinum*), A.p. (*Acer platanoides*), I.n. (*Impatiens noli tangere*), M.a. (*Mercurialis annua*), F.e. (*Fraxinus excelsior*).

layer. Such ideas are supported by experimental studies as summarized in the review of Terashima and Hikosaka (1995). So again, the upper cells do most photosynthesis and the lower cells the least. This is so regardless of whether the overall intensity is high or low. What this means is that as the light intensity increases the photosynthesis of all the layers goes up in parallel, and in proportion, so that they hit saturation at the same external light intensity. So while it should be the case that, in low light, the surface areas of the chloroplasts in the upper part of the leaf preferentially contribute to g_m, the same result should also be true at high, saturating light intensity and thus g_m should be comparable with changing A.

In order to explore the interaction between mesophyll conductance for CO_2 and water properties, we used the effective pathlength L as a proxy for changes in mesophyll water pathways [Ferrio et al. (2012)]. L was obtained from fitting a steady state evaporative enrichment model to measured lamina leaf water $\Delta^{18}O_L$ in the afternoon when steady state conditions were assumed. The comparison between the steady state (Δ_{LsP}) and the non-steady state model (Δ_{LnP}) indicates that isotopic non-steady state only needs to be considered directly before and during the night. This finding is in good agreement with recent observations [Cernusak et al. (2005), Farquhar and Cernusak (2005), Cuntz

et al. (2007), Gessler et al. (2013)] and corroborates our postulate that L can be fitted at 15:00 h under steady state assumptions. The values obtained for L are within or close to the range given usually in the literature [0.4–17 cm according to Wang et al. (1998)], with the exception of A. ursinum (but see below).

L increased clearly in the drought treatment in *I. noli tangere*, *A. ursinum* and *A. platanoides* but was not significantly affected in *M. annua* and *F. excelsior* . Observations of the response to reduced water supply among different species have included both an increase [Ferrio et al. (2009), Ferrio et al. (2012)] and no significant change of L [Kahmen et al. (2009)]. Only recently it was found that L is strongly dependent on transpiration at low E (below approximately 0.5–3 mmol $m^{-2}s^{-1}$) but not at E above this threshold value [Ferrio et al. (2012), Song et al. (2013)]. Consequently, the different response of L toward drought in different species can be explained by the extent of drought-induced changes in E. As pointed out by Ferrio et al. (2009), the underlying mechanism for such relationships could be a shift in water pathways (apoplastic/symplastic/transcellular), but also could be a reduction in the number of functional pathways, affecting both hydraulic conductivity and path tortuosity [Morillon and Chrispeels (2001), Pou et al. (2013)]. Indeed, the lack of differences in L found by Kahmen et al. (2009) coincided with no drought response in leaf hydraulic conductivity, despite significant changes in g_s and E. In contrast, in Ferrio et al. (2012) L was better correlated to leaf hydraulic conductivity than to E. Nevertheless, due to uncertainties associated with the calculation of L, together with covariation of many variables in drought response, a mechanistic explanation for changes in L is still a controversial issue [Zhou et al. (2011), Ferrio et al. (2012), Cernusak and Kahmen (2013), Song et al. (2013)].

In our study we also observed an exponential relationship between E and L with a strong increase in L for E <0.5 mmol $m^{-2}s^{-1}$ comparable to the observations of Ferrio et al. (2012) and Song et al. (2013). This finding can also explain the generally high L values observed in *A. ursinum* as in this species transpiration rates during the diel course experiments were low (Fig. 6). Recently Song et al. (2013) observed comparably high values for L at very low transpiration rates in pine species. We should note in this context that understory species such as the ones examined in our study generally show low transpiration rates [Givnish (1988)]. Moreover, understory species might behave similarly to shade- leaves, which appear to have stronger limitations from g_m [Tosens et al. (2012), Cano et al. (2013)].

In contrast to Ferrio et al. (2012), we found no clear relationship between g_m and L. This might be attributed to the fact that Ferrio et al. (2012) examined only the effects of drought on one species (Vitis vinifera), which is rather adapted to high light whereas

we assessed the variations across shade-tolerant understory species. According to Tosens et al. (2012), leaves developing under low light show most CO_2 limitations at the stroma, a compartment that is not shared by water movement, and show less contribution of diffusion limitations at the cell wall and across membranes than light-adapted leaves. Thus, potentially shade-leaves would tend to have less common pathways for CO_2 andwater through the mesophyll. Moreover, the drought conditions applied by Ferrio et al. (2012) were more severe than the ones applied here, as indicated by the much stronger reduction of g_s.

Our results indicate that an increase in g_m does not automatically imply a reduction in L and, subsequently, not in the tortuosity of the water movement in the mesophyll either. On the basis of the results presented here we have to reject our working hypothesis that the tortuosity for leaf water movement (L) in general scales inversely with g_m. We might, however, speculate that more severe drought stress as applied by Ferrio et al. (2012) might cause a stronger interference between the pathways of water and CO_2 movement and also that inter-specific difference or differences between functional plant groups (e.g. light- vs dark-adapted species) might define the degree of interference. There are two main explanations for the lack of a clear correlation of mesophyll CO_2 and water conductance across the five species under our experimental conditions: (1) Under the rather mild drought we applied, trans-membrane diffusion of water does not play a large role for water flux in the five species selected. If water is mainly transported via the apoplastic pathway any change in the permeability of membranes will thus neither affect the effective pathlength L nor hydraulic conductivity. (2) Aquaporin-mediated CO_2 and H_2O transport are independent or even competing. As suggested by Otto et al. (2010), aquaporins form heterotetramers in the membranes, and depending on the monomer composition a pore either facilitates water or CO_2 transport. Ferrio et al. (2012) gave a mechanistic explanation for the fact that both (1) increasing CO_2 conductance and declining water conductance (high L) and (2) increasing CO_2 and water conductance have been seen in experiments. The authors assumed that positive correlations between g_m and the path length of mesophyll water transfer were due to altered expression of a distinct aquaporin class changing the proportion of the monomer types in the heterotetramers. In other words, an increase in the relative proportion of the PIP1 aquaporin facilitating CO_2 transport would increase g_m and at the same time reduce the membrane permeability for water. Negative correlations between g_m and L might be due to variations in total aquaporin expression with no changes in the composition of the heterotetramers. For the species we tested such a joint increase in g_m and L upon drought was observed in *A. ursinum* and *I. noli tangere*. In the other species either a (slightly)

negative relationship could be observed (*F. excelsior*, *M. annua*) or an increase in L with no change in g_m (*A. platanoides*) was observed. We might thus speculate that different regulation of aquaporin expression and tetramer composition among species leads to the lack of a consistent trend between g_m and L.

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Chapter II

Impact of interspecific competition and drought on the allocation of new assimilates in trees

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Abstract

In trees, the interplay between reduced carbon assimilation and the inability to transport carbohydrates to the sites of demand under drought might be one of the mechanisms leading to carbon starvation. However, we largely lack knowledge on how drought effects on new assimilate allocation differ between species with different drought sensitivities and how these effects are modified by interspecific competition. We assessed the fate of ^{13}C labelled assimilates in above- and belowground plant organs and in root/rhizosphere respired CO_2 in saplings of drought-tolerant Norway maple (*Acer platanoides*) and drought-sensitive European beech (*Fagus sylvatica*) exposed to moderate drought, either in mono- or mixed culture. While drought reduced stomatal conductance and photosynthesis rates in both species, both maintained assimilate transport belowground. Beech even allocated more new assimilate to the roots under moderate drought compared to non-limited water supply conditions, and this pattern was even more pronounced under interspecific competition. Even though maple was a superior competitor compared to beech under non-limited soil water conditions, as indicated by

the changes in above- and belowground biomass of both species in the interspecific competition treatments, we can state that beech was still able to efficiently allocate new assimilate belowground under combined drought and interspecific competition. This might be seen as a strategy to maintain root osmotic potential and to prioritise root functioning. Our results thus show that beech tolerates moderate drought stress plus competition without losing its ability to supply belowground tissues. It remains to be explored in future work if this strategy is also valid during long-term drought exposure.

Introduction

Current climate models predict that numerous important forest regions in Central Europe will experience increasing frequencies and severities of drought periods [IPCC (2007),Christensen et al. (2007)]. The expected drier climatic conditions will not only have consequences for photosynthesis and primary productivity [Ciais et al. (2005), Granier et al. (2007)], but will also affect plant carbon allocation dynamics [Epron et al. (2012), Bahn et al. (2013), Zang et al. (2014)] with major impacts on the global C balance of terrestrial ecosystems. In trees, the interplay between reduced carbon assimilation and the inability to transport carbohydrates to the sites of demand is presently discussed as one of the mechanisms leading to carbon starvation and finally tree die-back under drought [Sala et al. (2010)].

Numerous studies have revealed a rapid and close link between photosynthesis and energy demanding processes especially in roots and rhizosphere [Högberg et al. (2001), Högberg et al. (2008), Högberg et al. (2010), Epron et al. (2011)] and thus the functioning of the belowground compartment in forest ecosystems strongly depends on the transfer of new photosynthates.

Moreover, it is necessary to consider the fate of newly assimilated carbohydrates in different plant tissues as well as the long-distance transport via the phloem [Paterson et al. (2009), Ruehr et al. (2009), Offermann et al. (2011)] to understand the biomass allocation dynamics as well as the root functioning in plants under changing environmental conditions [Blessing et al. (2015)].

It is widely acknowledged, that new assimilates are preferably transferred to tissues with the highest demand [Lambers et al. (2008)]. For example, under light limitation, plants tend to transport higher amounts of photosynthates to aboveground organs, whereas, under reduced nutrient availability or drought, they can allocate more carbon to the root system [Bloom et al. (1985), Kobe et al. (2010), Poorter et al. (2012)]. In their review, Poorter et al. (2012) indicate that moderate water stress causes only a small increase in

the root mass fraction (i.e., the relative contribution of root biomass to the total biomass) of plants and thus only a slight increase in belowground carbon allocation. Under severe drought, however, the root mass fraction strongly increases [Arend et al. (2011), Kuster et al. (2013)]. This is in contrast to assessments on the transport of recent assimilates belowground as Ruehr et al. (2009) showed that the amount of carbohydrates arriving in the belowground compartments strongly decreased in drought sensitive European beech under severe drought. Moreover, Meier and Leuschner [Meier and Leuschner (2008b)] report a strong reduction of fine root biomass in European beech under reduced soil water supply due to not only a shorter fine root lifespan but also to a 10-fold reduction in growth rates. Thus, there seems to be no unequivocal plant carbon partitioning and assimilate allocation response to drought and more information is necessary to understand allocation priorities and mechanisms in plants under environmental stress.

Pulse labelling approaches, applying either ^{13}C or ^{14}C enriched CO_2, allow for tracing the allocation of recent assimilates to respiration, growth, and storage pools [Epron et al. (2012), Carbone et al. (2007), Kagawa et al. (2006), Hartmann et al. (2015)] and thus are highly suitable tools to assess the mechanistic basis of short-term carbon partitioning in plants and between plants and the rhizosphere.

There is some information on how changing environmental conditions, such as reduced water availability, affect these short-term dynamics of carbon fluxes and patterns of assimilate distribution within trees (e.g. [Ruehr et al. (2009)]). For the rather drought sensitive European beech, soil water restriction not only decreases the amount of new assimilates transferred belowground but also increases transport times considerably [Zang et al. (2014), Barthel et al. (2011)]. Nevertheless, we largely lack knowledge on how drought effects differ between species with different drought sensitivity and how they are modified by inter-specific interactions.

It is generally known that interspecific interactions are able to change biomass allocation to fine roots of trees leading either to a reduction of fine root biomass [Schmid and Kazda (2002), Brandtberg et al. (2000)] or to increased fine root growth [Leuschner et al. (2001)], depending on the competitive abilities of the species involved. There is, however, no information on how plant species interaction changes short term sink priorities as assessed by tracing isotopically labelled assimilates.

The biosphere mediates the carbon flow from the atmosphere to the soil system also on short time scales [Barthel et al. (2011), Blessing et al. (2015)] and in the future the frequency of extreme events such as drought will most likely increase. It is thus important to understand the mechanisms that drive carbon allocation, carbon transfer times and above-to-belowground coupling strength as affected by environmental drivers. Moreover,

forestry in Central Europe, as well as in many other regions world-wide, promotes mixed-species stands that are assumed to be more resistant to environmental stressors [Millar et al. (2007), Pretzsch et al. (2013)] and to provide higher levels of multiple ecosystem services [Gamfeldt et al. (2013)]. As a consequence, insights concerning the effects of not only abiotic drivers but also species interaction on mechanisms that control carbon allocation within trees are crucial.

The present study aims to contribute to the understanding of the plant physiological mechanisms affecting key parameters of carbohydrate assimilate transport in trees under water restriction and inter-specific interaction. We assessed the fate of ^{13}C labelled assimilates in above- and belowground plant organs and in root/rhizosphere respired CO_2 in two tree species. Saplings of Norway maple (*Acer platanoides*), known to be rather drought tolerant [Webster et al. (2005), Linder (2000)] and European beech (*Fagus sylvatica*), assumed to be sensitive to reduced water availability [Geßler et al. (2007)], were exposed to restricted soil water supply either in mono- or in mixed culture. We exposed the trees to moderate drought as normally expected in the forest understory, in which extreme conditions (particularly soil moisture, air humidity and temperature) are normally buffered by the overstory canopy [Fotelli et al. (2003)].

We hypothesized that (1) drought would in general lead to a clear reduction of assimilate transport belowground and thus a lower ^{13}C signal in respired soil respired CO_2 (i.e. in CO_2 mainly originating from root/rhizosphere respiration). Furthermore, we expected a clear drought-mediated increase in the mean residence time (MRT) of new assimilates in leaves and the delayed appearance of the ^{13}C label in root organic matter and soil respiration. We hypothesized these effects to be stronger in the drought sensitive beech compared to the more drought tolerant maple. Moreover, we assumed that (2) in the mixed-species treatment, Norway maple would be able to outcompete the drought sensitive beech under water restriction mainly by taking advantage of the impairment of beech to allocate newly assimilated carbon belowground. As a consequence, we also expected an increase in belowground carbon allocation in Norway maple under drought when interacting with beech.

Materials and methods

Plant material and experimental set up

The study was conducted in a greenhouse with controlled air temperature (20±2°C). To simulate a diel cycle with a light period of 15h, additional artificial illumination

was applied (bulb type NARVA NC 1000–00) and the photosynthetic photon flux density (PPFD) at the canopy level was not less than 600 μmol $m^{-2}s^{-1}$. Two different tree species typical for mixed deciduous forests in Central Europe were selected for the experiment. *Fagus sylvatica* (European beech) is the most abundant species of the potential natural vegetation in central Europe. Owing to its sensitivity towards low water availability [Ellenberg and Leuschner (1996)] and longer drought periods [Fotelli et al. (2002)], physiological performance, growth and competitive ability of European beech may be adversely affected under future climatic conditions [Rennenberg et al. (2004)]. *Acer platanoides* (Norway maple) has a natural distribution area spanning from Scandinavia and Russia to Southern France and Turkey. It is known to have high water use efficiency (WUE) [Kloeppel and Abrams (1995), Hommel et al. (2014)] and is considered to be relatively drought tolerant [Linder (2000)].

The tree saplings were obtained from a commercial tree nursery (Handel, Metzingen, Germany). The 2-year-old seedlings were planted in 60 l plant tubs filled with organic, peaty container substrate (Kausek, Mittenwalde, Germany) before leaves were developed. Moreover, 50 g of lime (Rüdersdorf, Germany) were applied per tub. 10 individuals were planted in a tub and the average distance between individuals was approx. 5-10 cm. Three interaction treatments were established by planting 1) Norway maple in monoculture, 2) European beech in monoculture, and 3) maple and beech in mixed culture (5 individuals of each species per tub; randomly planted, Fig 22S). There were 20 tubes per interaction treatment resulting in 200 trees per treatment, and 600 trees in total.

The bulk density of the organic soil substrate was 0.12±0.03 g cm^{-3}. Volumetric soil water content (θ_s) was monitored continuously with EC-5 soil moisture sensors (Decagon Devices Pullman WA, US) at a depth of 10-15 cm (Table Z). Soil water potential (Ψ_s) was derived from θ_s according to [Schindler et al. (2010)]. During the acclimation period in the greenhouse, the tubs containing the plants were watered every second day to field capacity. After 3 months of growth in the greenhouse, half of the tubs of each interaction treatment (30 tubs in total) were exposed to moderate soil water restriction (drought treatment). This drought treatment started after the leaves fully developed.

We lowered Ψ_s to the thresholds of soil moisture tension (-0.21 to -0.24 MPa) for forest tree species [Bittner et al. (2010)] and these thresholds were reached after a couple of days without watering. When falling below the thresholds, pots were carefully watered to reach again these target values. The achieved thresholds in soil moisture imply a minimal root water uptake as defined by Bittner et al. (2010)]; below the threshold value root water uptake is assumed to be zero. By adjusting Ψ_s to the minimal water uptake

threshold we exposed plants to moderate drought stress but avoided damage or death due to severe drought conditions [Hommel et al. (2014)].
The other half of the tubs was well-watered by maintaining soil moisture at field capacity (control treatment). On day 14 of the drought treatment, the plants were pulse labelled with $^{13}CO_2$. Volumetric soil water content was between 44 % and 50 % in the controls and between 26 % and 33 % at the end of the drought treatment (on day 18 after the onset of drought equalling day 4 after $^{13}CO_2$ labelling).

Table 2: Characterization of the species and the experimental conditions. Plant age is given in years and the leaf-, stem- and root biomass in gram (g) dry weight per plant. Tree height at the beginning of the experiment and number of harvest times in each scenario is also given. At each harvest, 3 plants per treatment and species were sampled. θ_s is the average volumetric water content (±SD) of the control (n=6) and drought (n=6) treatment during the whole drought treatment period. Leaf-, stem- and root biomass is given as average of the treatment (control/drought) of all trees at all harvest time points (monocultures n=18, mixed n=12). Statistical differences in plant biomass was assessed by GLM ANOVA. n.s.: not significant.

	Monocultures		Mixed	
Species	Maple	Beech	Maple	Beech
Age (years)	2	2	2	2
Height (cm)	40-80	30-50	40-80	30-50
θ_s (%)				
Control	47.9±0.1	43.8±2.0	50.0±0.1	50.0±0.1
Drought	33.0±0.1	25.6±3.0	27.7±0.1	27.7±0.1
Leaf Biomass (g)				
Control	2.77±1.99	4.33±1.88	9.84±4.42	1.69±0.60
Drought	4.75±2.51	3.11±0.85	5.28±3.44	1.53±0.56
Stem Biomass (g)				
Control	4.84±1.63	8.97±3.45	15.09±9.87	4.07±1.71
Drought	5.59±2.87	5.29±1.73	9.43±5.34	3.07±1.47
Root Biomass (g)				
Control	3.38±1.85	8.81±4.41	5.75±2.82	4.91±2.08
Drought	2.66±1.36	7.97±4.82	6.01±3.46	4.75±1.30
Harvests	6	6	4	4

Determination of δ^{13}C in soil CO_2

For each interaction treatment, 7.0 x 2.5 cm sintered glass diffusers (Antstore Europe, Berlin, Germany) with gas-permeable porous walls were installed before planting the trees horizontally at depths of 20-25 cm into the soil. The diffusers were connected with gas tight PTFE tubing to transfer the soil gas via a multiport Valco valve sequencer (Vici, Houston, USA) to a cavity ring-down isotopic laser spectrometer (G2101-i Picarro Isotopic CO_2 Analyzer; Sunnyvale, USA). Flow rates for all tubes were adjusted via pumps to 100 L per h controlled by flow meters and needle valves. The soil gas $^{12}CO_2$ and $^{13}CO_2$ concentrations (and thus the δ^{13}C) were sequentially measured for all six tubs. Each tub was connected for 50 min to the laser spectrometer, followed by the measurement of the CO_2 concentration of the ambient air for 10 min. The laser spectrometer was calibrated before and after the experiment. The drift was less than 1‰ which is negligible compared to the strong δ^{13}C enrichment achieved in the labelling experiment.

$^{13}CO_2$ pulse labelling

18 hours prior to the $^{13}CO_2$ labelling, the belowground compartment was separated and made gas tight from the aboveground compartment by coating the soil surface with liquid silicone (TACOSIL 145, Dresden, Germany). We bored holes in the sidewall of the tubs to prevent anaerobic conditions in the soil compartment. Prior to labelling, transparent chambers (0.48 m x 0.74 m x 1.5 m; volume 280 L) were placed over each tub and sealed gas tight. The $^{12}CO_2$ concentrations within 6 chambers were measured simultaneously with portable gas analysers (IRGA; Li-820, Lincoln, NE, USA).
^{13}C sodium bicarbonate powder was dissolved in distilled water and added to the chamber by using a general injection needle. A beaker filled with phosphorus acid (H_3PO_4) was placed inside each chamber and the dissolved label (0.3 g 99 % ^{13}C sodium bicarbonate per tub) was injected into the acid to release $^{13}CO_2$ as soon as CO_2 concentration inside the chamber approached approx. 100 ppm. The whole $^{13}CO_2$ released from the bicarbonate within a few minutes equalled a concentration within the transparent chamber of 310 ppm. Plants were thus exposed to approx. 410 ppm CO_2 (c. 310 ppm $^{13}CO_2$, and c. 100 ppm $^{12}CO_2$) at the beginning of the labelling experiment. Two hours after labelling start, the chamber and the silicon layer separating the soil and the aboveground compartment were removed. During labelling, the air temperature increased by not more than 5°C in all treatments.

Harvest and isotopic analyses

Plants were harvested 24h before pulse labelling as well as 2h (immediately at labelling stop), 24h, 48h and 96h after the start of the $^{13}CO_2$ labelling. At each harvest time point, 3 plants per treatment and species were sampled from different tubs. Furthermore, for each harvested plant, assimilation rate (A), transpiration rate (E) and stomatal conductance (g_s) were measured with a portable infrared gas-exchange analyser (GFS-3000; Walz, Effeltrich, Germany) directly before sampling.

In order to avoid disturbing the $^{13}CO_2$ soil gas analyses by plant removal due to harvest sampling, we excluded 6 of the 20 tubs (3 drought, 3 control) from each interaction treatment from destructive harvest and used these exclusively for the online CO_2 measurements whereas the remaining 14 tubs were used only for plant harvest. Each harvested plant was separated into leaves, upper phloem (3 cm below the upper end of the main stem) and associated xylem, lower phloem at the stem base (3 cm above soil surface) and associated xylem as well as roots. The whole biomass of the different fresh plant organs was determined directly after harvest and an aliquot was dried at 75°C in the oven until the mass was constant to determine dry weight.

A subset of the harvested plant material was placed in plastic tubes and immediately frozen in liquid nitrogen and stored at -30°C. Afterwards, these samples were ground in liquid nitrogen and water soluble organic matter (WSOM) was extracted for the analysis of the carbon isotopic composition and carbon content according to [Ruehr et al. (2009) and Gessler et al. (2009)]. For the water extraction, 70±5 mg of leaf, phloem and xylem fresh material, and 140±5 mg of fresh root fresh material was used.

Ground plant material was shaken at 4°C with deionized water for 1h by using an incubator (Enviro Genie SL1200, USA). Afterwards samples were heated (Thermomixer, Eppendorf, Germany) to precipitate soluble proteins and centrifuged twice. An aliquot of each supernatant was transferred to tin capsules and dried at 30°C (Concentrator Plus, Eppendorf, Germany).

$\delta^{13}C$ in the dried extracts was determined by combusting the samples in a Flash HT elemental analyser (ThermoFinnigan) coupled via a ConFlo III interface to a Delta V advantage isotope ratio mass spectrometer. The precision of the measurement was <0.1‰. The isotopic values are expressed in delta notation (‰ units), relative to VPDB (Vienna Pee Dee Belemnite).

To estimate the amount of ^{13}C added by pulse labelling to the WSOM pool of the leaves and the roots, the $\delta^{13}C$ values were converted to atom% ^{13}C. We then calculated the

^{13}C excess, i.e. the increase in the amount of ^{13}C of the WSOM pool due to the $^{13}CO_2$ exposure according to Ruehr et al. (2009), based on the total leaf and root biomass of a single plant for the time point of maximum ^{13}C incorporation in the given tissue. Furthermore, we calculated intrinsic water use efficiency (iWUE) from gas-exchange (iWUE = A/g_s) at each harvest.

We computed the mean residence time (MRT) of WSOM in leaves as well as for soil $^{13}CO_2$. We acknowledge that besides transport processes in the plant, differences in soil diffusivity in the different treatments might have affected the temporal pattern of the ^{13}C label in soil CO_2. Since we, however, collected the CO_2 directly in the rooting zone we assumed this effect to be negligible. The mean residence time corresponds to the carbon stock to carbon flux ratio [Epron et al. (2012)] and was calculated by fitting the following exponential decay function.

$$N(t) = N_o e^{-\Lambda t} \tag{11}$$

Where t is the time in hours after labelling; N_o is the initial quantity of δ^{13}C at time t=0 (^{13}C peak); Λ is the decay constant; and N(t) is the quantity of ^{13}C after time t. The mean residence time (in hours) was then calculated as MRT = 1/ Λ.

Statistical analyses

All measured variables (in the drought and the control treatment) were tested for normality and characterized by descriptive statistics (means and standard deviations of the means). Effects of species, drought treatment and competition treatment on biomass, photosynthesis, stomatal conductance, ^{13}C excess, and MRT for leaf WSOM were assessed by analysis of variance (GLM ANOVA). For analysis, species was nested within competition treatment. To compare the time courses of δ^{13}C in WSOM of different tissues we applied a factorial repeated measure ANOVA where species was nested in the competition treatment. For δ^{13}C in soil CO_2 we only compared drought and well watered control for each the individual competition treatment by repeated measure ANOVA and MRT for soil CO_2 of drought and well watered control were compared by student's t-test. The error bars in the figures represent the standard deviation (SD). Statistical analyses and fittings were carried out with R 2.8.0 (R Development Core Team 2010), [[Pinheiro et al. (2008)], NCSS 2004 (Number Cruncher Statistical Systems, Kaysville, USA) and SIGMA-PLOT 12.3.

Results

Biomass analyses and plant gas-exchange measurements

Leaf biomass per individual plant for the two monocultures and the mixed culture was not significantly affected by the drought treatment (Tab. 2). Competition affected leaf biomass and a significant interaction between species and competition was observed, leading to higher values in inter- compared to intra-specific competition in beech and opposite patterns in maple. There was no significant effect of drought on stem and root biomass. For stem biomass a species and competition effect as well as an interaction between the two factors was detected. Comparable to leaves, stem and root biomass increased under interspecific competition in maple and decreased in beech.
Assimilation (A) was significantly reduced by drought. Moreover, a species effect was detected and interspecific competition led to a general reduction of A compared to intraspecific. As observed for A, g_s was reduced by drought. In addition, g_s values were generally lower in maple compared to beech (Fig. 8).

δ^{13}C in soil respired CO_2

We followed the time course of the δ^{13}C in soil respired CO_2 after the label pulse to assess the intensity and the speed of the linkage between photosynthesis and energy demanding processes belowground [Kayler et al. (2010)]. We acknowledge that the CO_2 derived from the soil is a mixture originating from autotrophic and heterotrophic respiration; the increase in the ^{13}C signal after pulse labelling, however, can be mainly attributed to root and rhizosphere respiration. All δ^{13}C values in the extracted soil CO_2 before label application were below -20‰ and thus well within the range observed for soil respiration [Werner and Gessler (2011)] indicating that dilution of soil respired CO_2 by ambient atmospheric CO_2 was negligible.
The first increase in δ^{13}C after labelling, i.e. the first appearance of ^{13}C tracer was detected in all water availability and interaction treatments after approx. 10 hours (Fig. 9).
It is evident from Fig. 9A that drought had neither a clear effect on the peak height nor on the slope of the curve in maple grown in monoculture. There were no significant differences in $\delta^{13}CO_2$ at any time point between drought exposed and control trees and the mean residence time was unaffected by drought (Tab. 3). Nevertheless, the $\delta^{13}CO_2$

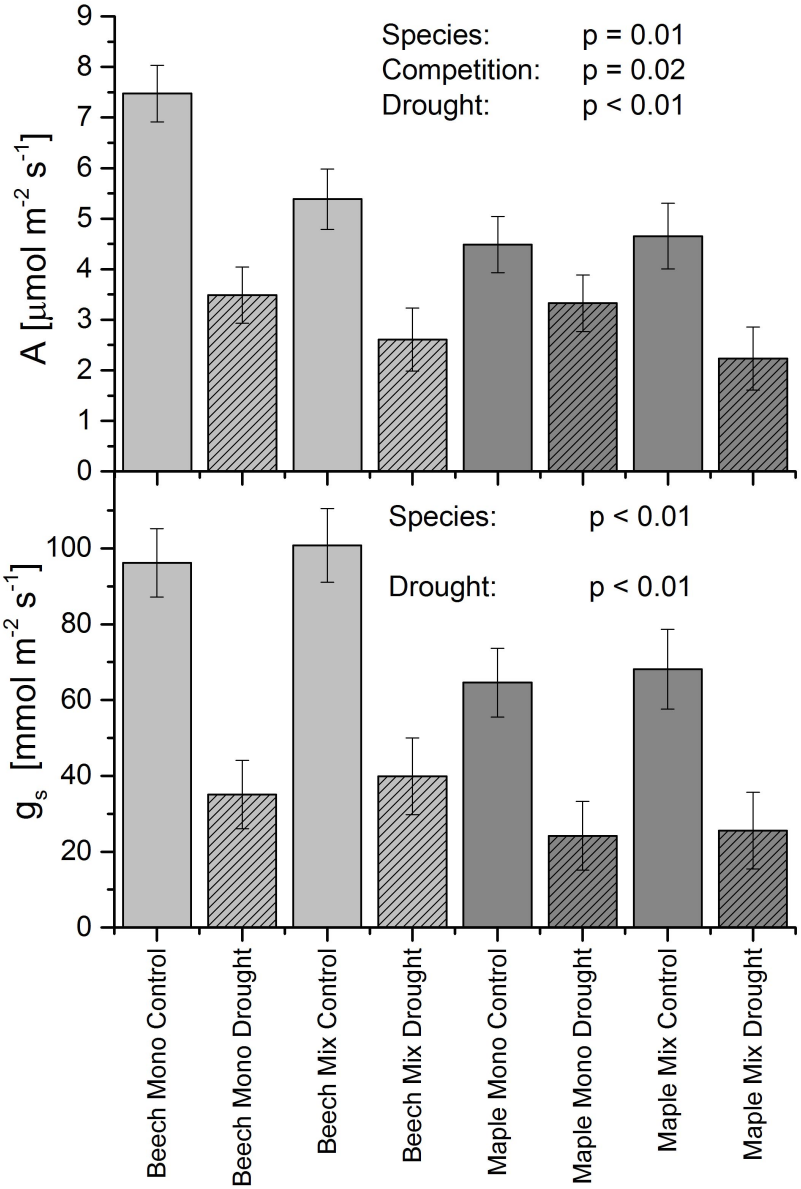

Figure 8: Assimilation rate (A) and stomatal conductance (g_s) of beech and maple in the drought and competition treatments. Values are given before 2h after the canopy ^{13}C labelling and the equivalent time points after the onset of drought (in days) are given on the upper x-axis. Data shown are from the drought (n=3) and control (n=3) treatments for monocultures and the mixed culture.

signal peaked earlier under drought; it was still increasing until approx. 26h in the control, when the $\delta^{13}CO_2$ signal under drought already started to decrease again.
In beech monocultures, drought significantly decreased the amount of ^{13}C label that arrived in the soil CO_2 (Fig. 9B). MRT in the beech monoculture control treatment was approx. 5-fold higher compared to the maple control. There was a tendency for MRT to increase upon drought in beech monocultures, but the difference between treatments was not significant (Tab. 3). The maximum label concentration arrived almost simultaneously in the two water availability treatments - between 34 and 38h - and thus slightly later than in maple.
In the mixed culture there was a tendency of a reduction in $\delta^{13}C$ of soil CO_2 compared to monocultures but significant decreases were only observed occasionally (Fig. 9C). Mean residence time remained unaffected by drought and was in-between the values for the two monocultures. The label maximum in CO_2 was detected after approx. 16h with no difference between the drought and the control treatment.

δ^{13}C of WSOM in the different plant organs

Leaves:
The maximum achieved $\delta^{13}C$ in leaf WSOM for both species in all treatments always occurred directly after the termination of labelling (equalling 2h after labelling onset; Fig. 3). In the monocultures of maple there was a slight though insignificant decrease in $\delta^{13}C$ of leaf WSOM upon drought at all harvest dates whereas beech showed the opposite pattern. In maple, this slight decrease is also reflected by the total amount of ^{13}C (given as ^{13}C excess per 20 plant in Table 3) determined in the foliage directly after labelling, which was in the same range in the two water availability treatments, though slightly lower under drought. The somewhat smaller ^{13}C excess in drought treated monoculture beech compared to controls is explained by the slightly smaller total leaf biomass (Tab. 2). Even though both species did not show significant changes in $\delta^{13}C$ of leaf WSOM as a reaction to drought at any harvest time point, the MRT increased significantly in beech monocultures (Tab. 3).
In the mixed cultures, the peak $\delta^{13}C$ in WSOM was smaller for both species compared to the respective monocultures and, in maple, drought significantly reduced $\delta^{13}C$ of WSOM at the first harvest time (Fig. 3). ^{13}C excess in the leaf WSOM for the whole plant 2h after labelling was thus decreased in the mixture for both species (Tab. 3). The decrease was small and insignificant for maple in the control treatment, due to the increase in

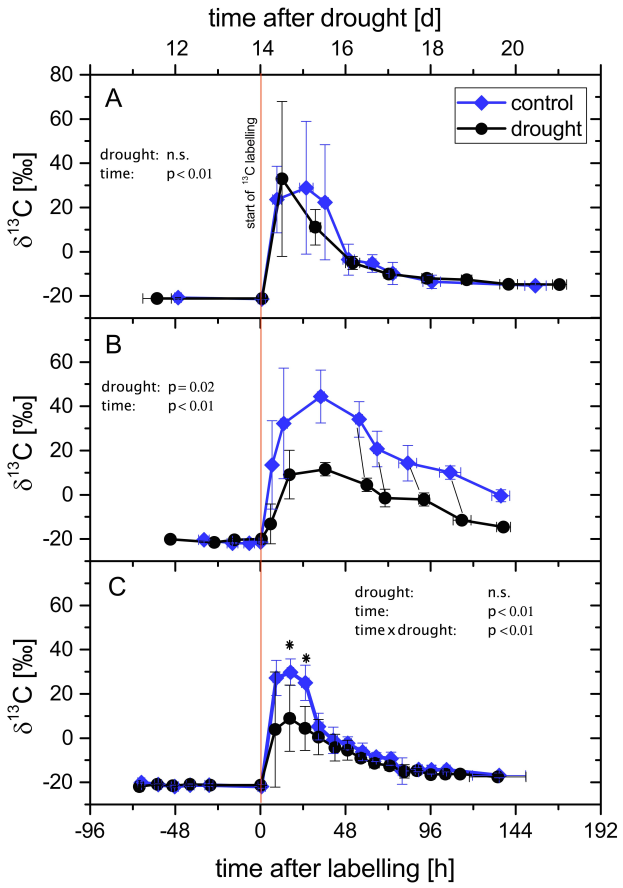

Figure 9: $\delta^{13}C$ in soil CO_2 after canopy ^{13}C label application. Blue symbols and lines indicate the control, and black symbols and lines drought treatments. $\delta^{13}C$ values of soil respired CO_2 in maple monoculture (A), beech monoculture (B) and mixed culture of maple and beech (C) are displayed. The $\delta^{13}C$ in soil CO_2 values were compared between drought and control for the different intra- and interspecific competition treatment by applying repeated measure ANOVA. n.s.: not significant. In (C) an additional test was carried out. The three first time points after labelling were compared (drought vs. control) by student's t-test; * denotes significant differences ($p \leq 0.05$) between drought and control for a given time point. The vertical solid red line indicates the start of the ^{13}C label application. In (B), the 4 black ledger lines indicate which time points were compared since there was a slight time shift between measurements of drought and control tubs. Horizontal and vertical error bars indicate SD.

leaf biomass levels of the mixed cultures compared to the monoculture (Tab. 2).

In both, the drought and the control treatment, MRT did not change significantly in the maple mixed culture compared to the monoculture. In beech, however, the mixed cul-

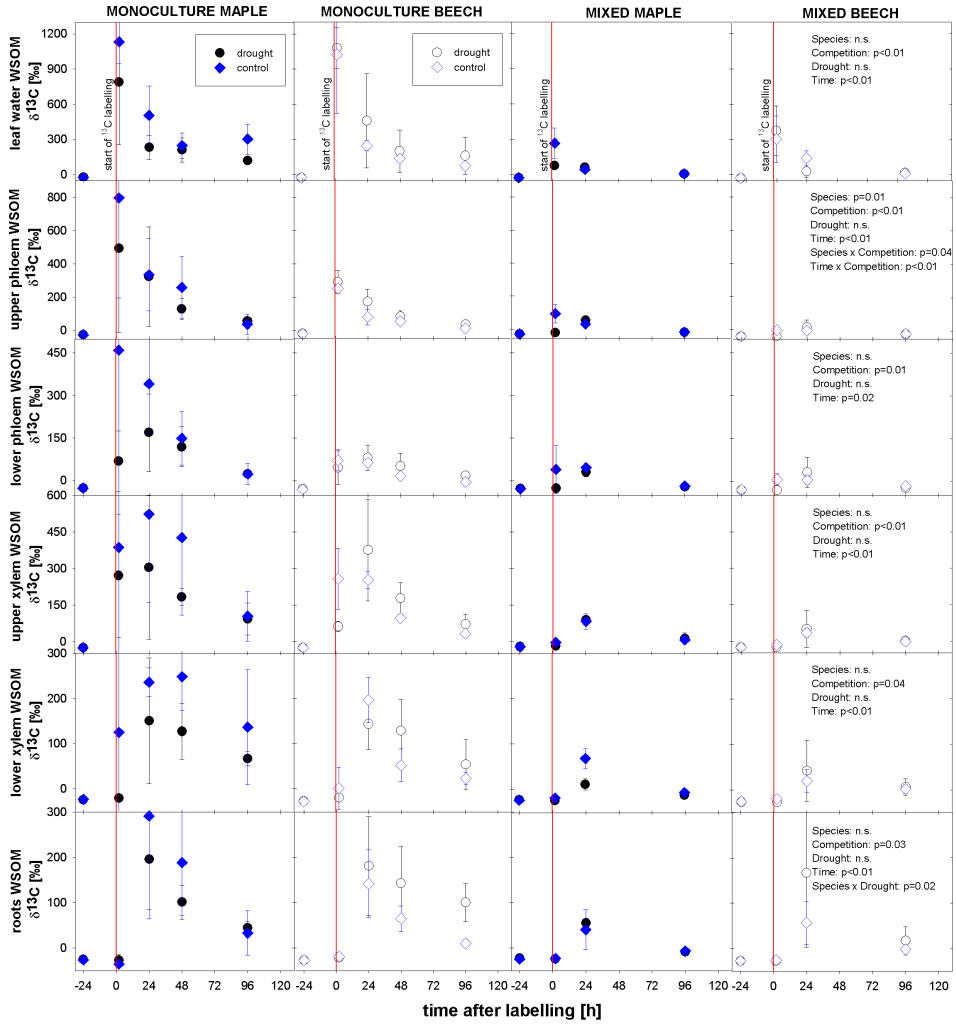

Figure 10: The four columns of graphs show beech and maple in different interaction treatments (monoculture, mixed) and the water availability treatments are given for a given interaction treatment by different colours (blue: control; black: drought treatment). The different rows of figures (from top to bottom) show δ^{13}C dynamics of WSOM in leaves, upper phloem, lower phloem, upper xylem, lower xylem and roots. The effects of species, drought, and competition treatment have been assessed by repeated measure ANOVA. The red line in each graph indicates the start of ^{13}C label application. Note that the y axis scaling is different for different plant organs.

ture MRT decreased under drought and increased under well-watered control conditions compared to the monocultures (Tab. 3).

Phloem and Xylem:
In the maple monoculture treatment, δ^{13}C in WSOM peaked 2h after labelling in the upper and lower stem phloem in the well watered control treatments, and δ^{13}C values were approx. 800 and 460‰, respectively (Fig. 10). In the upper and lower stem xylem the δ^{13}C peak occurred after 24h and 48h, respectively. δ^{13}C-WSOM values in phloem and xylem tended to decrease under drought, but the change was not statistically significant. In contrast, for maple within mixed cultures the maximum δ^{13}C in WSOM decreased significantly in the upper stem phloem and xylem in the well watered control treatments. At the lower stem the δ^{13}C-WSOM values were lower by trend at all harvest time points, but the differences between the monocultures and mixed cultures treatments were not significant under optimum water supply. In the mixed culture, drought decreased δ^{13}C 2h after the onset of the labelling in the maple phloem at both stem positions as well as the δ^{13}C peak at 24h in the xylem at the stem base.
In the well-watered controls of the beech monoculture, δ^{13}C in phloem WSOM peaked at 2h after labelling at both stem positions. The peak was slightly delayed in the xylem, spanning a time period from 2 to 24h. As in maple, drought did not reduce the δ^{13}C time course in beech monoculture. However, and in contrast to maple, there was a slight tendency for an increase in δ^{13}C-WSOM values as consequence of drought. Moreover, maximum δ^{13}C WSOM values were significantly reduced in phloem and xylem within the mixed cultures compared to the monocultures under high water supply. As in the monoculture, drought did not affect temporal δ^{13}C pattern in phloem and xylem in beech in the mixed cultures.
Roots:
δ^{13}C in root WSOM peaked after 24h in both species, independent from drought or culture-type (monoculture vs. mixed culture) (Fig. 3). Drought did not have a significant effect on the temporal patterns of δ^{13}C in roots of maple neither within mono- nor mixed cultures. In beech in contrast, δ^{13}C in root WSOM was higher as a result of drought. This difference was significant in monocultures at the end of the sampling period (96h after labelling) and in the mixed cultures at the time of the δ^{13}C peak 24h after the start of labelling. ^{13}C excess on a per plant basis after 24h (when the highest label in roots was detected) showed patterns comparable to δ^{13}C in WSOM with clearly higher values in beech roots in mixed cultures as a consequence of drought (Table 3). When relating the maximum ^{13}C excess in roots (after 24h) to the maximum excess in leaves (after 2h), the relative amount of ^{13}C transferred to the roots clearly increased for beech in monoculture as a consequence of drought (from 15% in the control to 28% in the drought treatment) and remained more or less constant for maple (5 to 6%). In

the mixed cultures the relative amount of ^{13}C transferred to the roots clearly increased upon drought for both species (maple: from 5% to 46%; beech: from 24% to 72%).

Table 3: ^{13}C excess and mean residence times. ^{13}C excess (μg/plant) is based on leaf and root biomass per average plant. ^{13}C excess values are given for the time point of maximum label in the given tissue. For leaves it was the harvest 2h after start of the ^{13}C labelling, for roots it was the harvest 24h after labelling. Mean residence time (MRT) is given in (h). Effects of species, drought and competition treatment as well as significant interaction as assessed with factorial ANOVA are given for tissues specific values. MRT for soil CO_2 of drought and well watered control were compared by student's t-test. Here, same letters indicate no significant differences.

	Monocultures		Mixed	
Species	Maple	Beech	Maple	Beech
^{13}C excess in leaves at ^{13}C peak				
Control	306.9±116.4	413.9±296.3	196.3±85.2	32.5±18.2
Drought	263.1±174.1	219.5±62.1	36.8 ±4.5	36.3±11.7
^{13}C excess in roots at ^{13}C peak				
Control	19.3±18.9	62.9±37.7	9.8±8.5	7.9±5.6
Drought	12.8±6.8	61.5±35.4	16.8±4.1	26.2±25.9
MRT in ^{13}C WSOM leaves				
Control	34.6±12.1h	17.3±3.1h	12.4±1.2h	28.5±0.4h
Drought	25.5±8.6h	28.3±5.2h	55.1±23.9h	8.3±2.1h
MRT of $\delta^{13}C$ of soil CO_2				
Control	14.1±4.4h^a	72.5±33.6h^a	27.3±3.8h^a	27.3±3.8h^a
Drought	17.1±1.7h^a	208.3±110.4h^a	56.5±22.6h^a	56.5±22.6h^a

Discussion

The effect of moderate drought on the allocation of new assimilates in monocultures

Based on recent studies on ^{13}C allocation [Zang et al. (2014), Ruehr et al. (2009), Barthel et al. (2011)] we hypothesized that drought would lead to a clear reduction in assimilate amount transported belowground and to a decrease in transport velocity in both species assessed here - but with a stronger effect for the drought sensitive beech. In contrast to the above mentioned studies, the drought intensity in our study was, however, rather moderate as we aimed at simulating the conditions in the forest understory where extreme environmental amplitudes are buffered by the sheltering canopy [Hommel et al. (2014), Gimbel et al. (2015), Felsmann et al. (2015)].

As expected, the moderate drought decreased assimilation rate in both species in monoculture, and the effect was more pronounced in drought sensitive beech (Fig. 1). This decrease in assimilation rate was, however, not reflected by the maximum $\delta^{13}C$ in leaf WSOM after ^{13}C labelling, which was not different between the two water availability treatments for both species (Fig. 3). WSOM is assumed to be reliable proxy for newly assimilated sugars [Brandes et al. (2006)]. Moreover, the ^{13}C excess in leaf WSOM, as a measure of the total uptake and conversion of $^{13}CO_2$ to soluble sugars, was also not significantly affected by the drought treatment (Tab. 3). This is in contrast to the observations of [Ruehr et al. (2009)] for beech, who found clearly higher maximum $\delta^{13}C$ and ^{13}C-excess in leaf WSOM in controls compared to drought-treated plants directly after the labelling. These authors, however, also showed that 2 days after the ^{13}C label application this difference was gone and they attributed their finding to more efficient and faster assimilate export from the leaf to the phloem under non-water limited control conditions. In our study, the maximum $\delta^{13}C$ value in leaf WSOM for both species was already detected at the first harvest, 2h after the beginning of labelling, i.e. directly after the ^{13}C exposure had stopped. We thus might speculate that at this time, significant amounts of ^{13}C labelled assimilates were already exported from the leaves (during the 2h of ^{13}C exposure) and that this – undetected amount – might have been greater in the non-water limited plants of the control treatment. This assumption is partially supported by the much faster increase in $\delta^{13}C$ in maple stem upper and lower phloem positions (Fig. 3) in controls compared to drought-treated plants and by the significantly higher leaf WSOM MRT in beech, indicating generally higher turn-over of new assimilates under well-watered control conditions.

Additionally, the putative mismatch between the reduced assimilation rate, but compa-

rable ^{13}C labels in leaf soluble sugars in the drought compared to the control treatment, could be explained by the faster use of new assimilates for storage and growth under well watered conditions. As the leaves of both species were fully developed, growth might play a minor part, but starch accumulation has been observed to be under environmental control. The division of assimilates between starch and sucrose is assumed to be internally regulated to suit the environmental conditions [Zeeman et al. (2007)]. It has also been observed that starch accumulates when the utilisation of newly produced triose-phosphates from the chloroplast becomes rate limiting to carbon assimilation, especially when photosynthesis and stomatal conductance are high [Beck and Ziegler (1989)]. Conversely, we might assume that the reduced photosynthesis observed in our drought treatment, for both species, might have led to less leaf starch accumulation compared to the controls. In agreement with this assumption, [Barthel et al. (2011)], showed that the mean residence time of ^{13}C labelled sugars in plants depends on the assimilation rate, in which higher assimilation rates resulted in faster turn-over, including the carbon utilized for respiration and storage.

While $\delta^{13}C$ values in phloem, xylem and root WSOM of maple under drought tended to decrease (although statistically insignificant), the opposite was observed for beech. In beech roots, $\delta^{13}C$ in WSOM significantly increased after 96h (Fig. 3). For both species, we did not observe a clear time lag in label transport, neither when considering WSOM nor soil CO_2 (Fig. 2).

While the first appearance of the label in soil CO_2 in the controls is comparable with result from beech and other tree seedlings [Epron et al. (2012), Barthel et al. (2011), Studer et al. (2014)], the absence of transport retardation upon drought is in contrast to previous findings [Zang et al. (2014), Ruehr et al. (2009), Barthel et al. (2011)]. Delays in assimilate transport from the leaves to roots for beech exposed to drought has been documented to range up to approx. 6-fold compared to controls [Ruehr et al. (2009)]. Moreover, these authors observed higher $\delta^{13}C$ values in root WSOM in the controls compared to drought exposed beech seedlings during the first 96h after ^{13}C exposure. Our findings indicate that drought intensity strongly affects assimilate allocation in trees: Under severe and longer-lasting drought as applied e.g. by [Ruehr et al. (2009)], transport to belowground tissues is reduced and retarded. While, under the more moderate drought conditions as applied in our study, transport of new assimilates is only slightly reduced (maple) or even slightly increased (beech), and no reduction of transport velocity occurs. Under moderate drought, an increase in carbon allocation belowground might be important to maintain root function, since reduced water availability increases fine root mortality and simultaneously stimulates compensatory fine root production [Meier

and Leuschner (2008a)]. However, drought intensity seems to modify trees ability to allocate new assimilates to roots, confirming previous observations that severe drought can strongly retard root growth while moderate drought can enhance carbon allocation to roots [Eghball and Maranville (1993)].
In maple, drought did not affect the amount of $\delta^{13}C$ label in soil CO_2 nor did it significantly change MRT (Fig. 2 and Tab. 3). This finding fits to the unchanged MRT for leaf WSOM and to the lack of significant drought effects on the $\delta^{13}C$ of WSOM in all plant organs. In contrast, beech exhibited significantly lower $\delta^{13}C$ values in soil CO_2 (after 48h and later) and a 2.9-fold higher MRT in the drought treatment. This pattern was accompanied by an increase in $\delta^{13}C$ in root WSOM. Beech is known to accumulate sugars as osmoprotectants, which increase osmotic pressure, maintain membrane integrity, and stabilize protein [Peuke et al. (2006)]. As a consequence, we can infer that new assimilates were utilized less intensively for respiration and other maintenance or growth-related processes.
In summary, we have to reject our first hypothesis: we found neither a significant reduction in the allocation of new assimilates belowground nor a significant reduction in transport velocity. The inability of trees to maintain phloem transport function under water limitation is seen as one of the main reasons for tree mortality due to carbon starvation [Sala et al. (2010)], but our results point to the fact that it is necessary to determine threshold values for drought intensity [Zang et al. (2014), Beier et al. (2012), Kayler et al. (2015)] to characterise when a system is tipping from one state (not impaired or even increased assimilate allocation under moderate drought) to another (impaired and retarded assimilate transport under severe drought).

The effect of drought plus interspecific interaction on the allocation of new assimilates

We initially hypothesized that the allocation of new assimilates to belowground tissues in mixed plantations would be strongly impaired in the drought-sensitive beech due to the combined effects of limited water availability and competition.
Under non-limited water supply, the interspecific interaction treatment led to a clear increase in biomass of all compartments (leaves, stems, roots) in maple and to a decrease in beech compared to the monospecific controls (Tab. 2). While the average assimilation rate of maple was not different between mono- and mixed cultures, amounting to 4.5 ± 2.0 μmol $m^{-2}s^{-1}$ during the observation period (Fig. 1), beech rates were slightly lower in the mixed culture (5.1 ± 1.6 μmol $m^{-2}s^{-1}$) compared to monocultures (7.5 ± 2.0

μmol $m^{-2}s^{-1}$). All these findings point to a competitive advantage of maple over beech under non-water limited conditions, which is in agreement with the results of [Simon et al. (2010)]. These authors found that beech was impaired in its nitrogen uptake when growing together with Sycamore maple, but maple in turn benefitted from the interaction and increased soil nitrogen uptake.

When water supply was not limiting, maximum leaf δ^{13}C-WSOM values for both beech and maple were lower in mixed cultures compared to monocultures (Fig. 3). The δ^{13}C patterns could not be explained by differences in leaf biomass between the interaction treatments since the absolute ^{13}C incorporation in leaf WSOM was also lower: With 10 individuals per tub, the total amount of ^{13}C recovered in WSOM 2h after labelling began was 3.07 mg ^{13}C for maple monocultures, 4.14 mg ^{13}C for beech monocultures, but only 1.15 mg ^{13}C in the mixed cultures (5 maple and 5 beech individuals). We might assume that in the interspecific interaction treatments, relatively more of the new ^{13}C labelled assimilates were incorporated into water insoluble storage compounds in leaves, stems and roots.

Similar to the monocultures, drought only slightly affected the δ^{13}C patterns of WSOM in the different organs of maple. The main difference might be seen in the significantly stronger initial ^{13}C signal of the control trees in the phloem 2h after the start of the label exposure (Fig. 3). This finding, along with the - though insignificant - increase in MRT of leaf WSOM under drought (Tab. 3), point to a drought-induced slowing down of phloem loading and transport for maple as previously observed for other species [Ruehr et al. (2009), Barthel et al. (2011)].

In contrast, MRT of beech leaf WSOM decreased during drought and δ^{13}C in root WSOM was significantly higher in the drought compared to the control treatment 24h after the start of labelling (Fig. 3). Moreover, drought led to a 3.4-fold increase in the maximum ^{13}C excess in roots. These results are partially in contrast to observations on the interaction between drought and competition on nitrogen uptake and allocation in beech [Fotelli et al. (2002)], in which drought not only increased the negative effects of the strong competitor Rubus fruticosus on beech ^{15}N uptake by the roots, but additionally led to impaired nitrogen allocation belowground. We, however, need to consider, that nitrogen allocation might be subjected to different environmental and internal controls than carbon transport and also that the moderate drought intensity applied in our study may not have been comparable to the other study.

With respect to carbon, in both species of our study a higher proportion of new assimilates was transported belowground under drought plus interspecific competition compared to (i) non-limited water supply plus interspecific competition and (ii) drought plus

monospecific interaction (i.e. monocultures).
We can state that maple was a superior competitor compared to beech under non-water limited conditions as indicated by the changes in biomass of both species in the interspecific interaction treatments as well as the clearly reduced total ^{13}C label incorporation in WSOM of beech (Tab. 3). However, as a consequence of our results, we have also to reject our second hypothesis: beech was still able to efficiently allocate new assimilates belowground under moderate drought and interspecific competition and even increased the relative amount in the mixed cultures compared to the monocultures. This might be seen as a strategy to maintain the root osmotic potential and to assert root function. Our short-term study, however, precludes us from predicting how moderate drought and intraspecific interaction would affect carbon allocation belowground and thus root growth and functioning in beech over the longer term.
In summary, our results clearly show that physiological responses of trees do not necessarily linearly follow the intensity of environmental drivers. In our case, a primary assumption was that the moderate drought applied here would have comparable though potentially weaker effects [Blessing et al. (2015)] compared to the stronger drought intensities applied in previous research [Ruehr et al. (2009)]. However, we neither saw a clear reduction of the allocation of new assimilates from the leaves to sink tissues nor did we detect strong indications for reduced transport velocity. On the contrary, beech allocated relatively more new assimilates belowground under moderate drought compared to non-limited water supply and this pattern was even more pronounced under interspecific competition. The drought induced inhibition of carbon transport within plants is crucial for understanding the mechanisms of carbon starvation, which is one of the most intensively discussed factors for tree mortality under water limitation [Sala et al. (2010), McDowell et al. (2008b)]. Recent studies [Beier et al. (2012), Kayler et al. (2015)] have called for new experiments with multiple levels of a given environmental driver to characterise trajectories and thresholds in species-specific responses. Our results strongly support this suggestion as it shows together with the previous work of Ruehr et al. (2009) and others that depending on the level of drought, allocation of recent assimilates belowground can be either stimulated or reduced.

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Chapter III

Stomatal conductance and intrinsic water use efficiency in the drought year 2003 - A case study of of European beech

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Abstract

We applied a hydraulic modeling together with a tree ring stable isotope approach in order to identify the physiological responses of beech trees to changing environmental conditions. The drought conditions of the extreme hot and dry summer in 2003 were hypothesized to significantly influence the radial growth of European beech mainly triggered by the stomatal response towards water scarcity leading in turn to a decline in carbon assimilation. The functional-structural single tree modeling approach applied, revealed in fact a strong limitation of water use and carbon gain during drought. However, tree ring width data did not show a clear drought response and no differentiation in radial growth during the six subsequent years examined in this study (2002 to 2007)

was observed. Using integrated results from mechanistic carbon-water balance simulations, tree ring carbon and oxygen isotope analysis and tree ring width measurements; we postulate that the suggested drought induced growth decline has been prevented by the remobilization of stored carbohydrates, an early onset in growth and the relatively late occurrence of the severe drought in 2003. Furthermore, we demonstrate that the stomatal response played a significant role in avoiding harmful water tension that would have caused xylem dysfunction. As a result of the combined investigation with physiological measurements (stable isotope approach) and hydrodynamic modeling of stomatal aperture, we could give insights into the physiological control of mature beech trees functioning under drought. We concluded that beech trees have operating at their hydraulic limits and that the longer or repeated drought periods would have affected the growth considerably.

Introduction

An increasing vulnerability of forests in many regions worldwide towards climate change induced drought stress has been described recently by various authors [Allen et al. (2010), Lindner et al. (2010), Anderegg et al. (2012), Choat et al. (2012), Cailleret et al. (2014), Rais et al. (2014), Anderegg et al. (2014), Doughty et al. (2014)]. Drought stress, e.g. induced by increased evaporation, has been identified as a reason for global forest dieback [Adams et al. (2009), Allen et al. (2010), Breshears et al. (2013)]. Moreover, even below the threshold of mortality, a general decline in productivity due to dry conditions can be expected from reduced assimilation and a relatively low sink priority for growth [Wiley and Helliker (2012)] or due to drought induced tissue damages decreasing their functionality and requiring additional carbon expenses for repair [Palacio et al. (2014)]. As an example, the extraordinary dry growing season in 2003 in Europe lead to an estimated reduction in gross primary productivity in ecosystems over Europe of about 30 % [Ciais et al. (2005)]. In forest ecosystems a reduction in annual radial growth has been observed in response to this drought year (e.g. Granier et al. 2007) but exceptions have also been noted [Hartl-Meier et al. (2014)]. In particular for European Beech (*Fagus sylvatica*), which is widespread in central Europe, growth decline has been demonstrated at various sites [Löw et al. (2006), Jump et al. (2006), Van der Werf et al. (2007), Charru et al. (2010), Maxime and Hendrik (2011)].

Two main hypotheses have been put forward to explain the reduction of tree growth and increased mortality: carbon starvation and hydraulic failure [McDowell et al. (2008a)]. Carbon starvation summarizes the situation when the carbon demand for maintenance

of cellular and defensive metabolism is not met owing to low carbohydrate supply from photosynthesis and storage [McDowell (2011)]. However, in several cases carbon starvation has been found to fail explaining growth decline and tree death [Körner (2003), Sala (2009)]. Nevertheless, the carbon supply status is likely to play an important role for repair and recovery as well as for secondary stress defense (Niinemets 2010). Hydraulic failure, however, seems to be the primary cause of plant mortality during drought due to xylem embolisms [Anderegg et al. (2012), Choat et al. (2012)] but might be avoided under high availability of mobile soluble carbon compounds [Adams et al. (2009), McDowell (2011), Gruber et al. (2012), Sevanto et al. (2014)].
While carbon starvation is expected to occur during prolonged drought periods in relatively isohydric plants closing their stomata at low xylem water tensions, hydraulic failure is expected to proceed more rapidly especially in relatively anisohydric plants keeping their stomata open during drought [Sevanto et al. (2014)]. However, because hydraulic functioning and carbohydrate and defense metabolism are strongly interdependent [McDowell (2011)], the explanation of growth decline and tree death may require a combination of these two hypotheses and supplementary explanations [Anderegg et al. (2012)]. While stomatal regulation in vascular plants is an efficient means for adjusting water use to changes in plant water supply and demand [Aranda et al. (2005), Sperry et al. (2003), Whitehead (1998)], the question how the impact on tree metabolism can be described considering a simultaneously reduced CO_2 uptake is still open [Sala et al. (2010), Zeppel et al. (2013)]. For example, tree species-specific variations in the mobilization of non-structural carbohydrates in response to water shortage and critical thresholds for storage compounds in trees may play an important role for the actual carbon supply during drought [Palacio et al. (2014)].
Because water shortage evokes a general predicament for plant gas exchange, i.e. the loss of water to gain carbon (Chaves et al. (2003)], the mechanistic description of this tradeoff is essential for the estimation of tree vulnerability to drought stress and of forest growth under future climate conditions. In addition, the representation of relations between carbohydrate availability and hydraulic regulation seems to be fundamental to describe long-term drought responses [Galiano et al. (2011), Mitchell et al. (2013), Sevanto et al. (2014)]. We aim to reveal which physiological responses occur and to which degree stomatal closure can be made responsible for growth declines during drought. Therefore we investigate mature trees of European beech and determined the ratio of water and CO2 exchange throughout years of contrasting water supply. The intrinsic water use efficiency (IWUE) as calculated by the ratio of the assimilation (A) and the stomatal conductance (g_s) is used here as an integrative measure of the carbon and water balance. IWUE

can be derived from the $\Delta^{13}C$ (carbon isotope discrimination) of tree rings allowing a retrospective view of the physiological responses under different environmental regimes [Farquhar et al. (1989) McCarroll and Loader (2004)]. The stable isotope composition of plant organic matter, in particular the carbon isotope ($\delta^{13}C$) and the oxygen isotope signatures ($\delta^{18}O$), has been widely used to address leaf level acclimation of A and gs to environmental drivers [Gessler et al. (2014) and Werner et al. (2012)].
Furthermore, the leaf water enrichment of ^{18}O depends on the ratio of atmospheric vapor pressure and intercellular leaf spaces [Dongmann et al. (1974)]. Thus, the $\delta^{18}O$ signature and the ^{18}O enrichment above source water ($\Delta^{18}O$) of tree rings can be associated to the stomatal sensitivity to changing evaporative conditions [Scheidegger et al. (2000)]. In order to capture the short- and long-term responses [Hentschel et al. (2014)], the tree ring isotope composition and the tree ring width were determined throughout a period of six subsequent years (2002 to 2007), which includes the extremely dry year 2003. Furthermore, we relate our results to the findings of a long-term growth assessment conducted at our study site [Poschenrieder et al. (2013)] covering the period between 1970 and 2010.
Measurements were applied to challenge the current knowledge about stomatal behavior and carbon assimilation by simulating gs based on a mechanistic, hydrodynamic modeling approach (Xylem Water Flow model, XWF). In the model, the hydraulic conductance of the soil-leaf continuum is functionally linked to the stomatal control of water loss-a fact that might furthermore dictate the limits of a species tolerance to water stress [Sperry et al. (2002)]. In the XWF model, gs is expressed as a function of leaf water potential which in turn depends on the evaporative demand and the water relations within the soil-leaf continuum [Bohrer et al. (2005)]. According to the cohesion-tension theory [Tyree and Zimmermann (2002)] and in consideration of tree anatomy and individual branching systems [Cruiziat et al. (2002)], the water transport in the soil-leaf continuum can be calculated on a solid physical basis [Hacke and Sperry (2001), Schulte and Brooks (2003), Tyree et al. (1994)]. Finally, we model the photosynthesis of the individual trees by using a bio-chemical approach established by Farquhar et al. (1980) which has been linked to the XWF model via its dependency on g_s.
Therefore, the individual hydraulic pathways of nine mature beech trees were parameterized from terrestrial laser scanning (TLS) images. Thus, the whole-tree hydraulic conductance could be linked with transpiration flux, carbon gain and growth rate. Since various authors have demonstrated a drought induced growth decline in European beech as mentioned above, we hypothesized for the drought in 2003, (i) that limited soil water supply should have caused a reduction of the annual radial growth as a major indication

of a distinct drought response. Moreover, we assumed (ii) that a mismatch between plant available water and evaporation demand occurred, leading to stomatal regulation and reduced H_2O and CO_2 exchange at the leaf level. As a consequence, we expected (iii) that a shift in the A-to-gs ratio towards increasing water use efficiency occurred. The first impact should be visible in the tree ring dimensions, the second should be reflected in the simulation of whole-tree hydraulic conductance, and the third in an increased IWUE.

Material and Methods

This study has been designed to examine the IWUE (μmol mol^{-1}) of individual trees derived by a hydrodynamic model approach (IWUEXWF) and a tree ring stable isotope approach (IWUEiso). We examined nine mature trees of a closed beech stand in Southwest Germany. The study trees ranged in diameter at breast height between 21.9 cm and 41.0 cm and in tree height between 25.4 m and 34.7 m. In order to examine the physiological response towards water shortage, we analyze six subsequent years of varying soil water supply (2002 to 2007) including the drought year 2003.
In order to evaluate the growth response of the trees, we additionally considered a long term growth assessment conducted at the same study site that included the nine beech trees mentioned above [Poschenrieder et al. (2013)]. These authors analyzed a stratified sample of 27 mature beech trees covering the years 1970 to 2010. The annual radial growth of the individual trees (DBHi) was determined by two tree cores taken with an angular distance of 90° from each other and one of the cores was taken from the uphill side.
Furthermore, Poschenrieder et al. (2013) contrasted their findings of the NE exposed study site with a similar growth analysis of a SW exposed beech stand in close vicinity. In order to compare the growth and the intrinsic water use efficiency at the two different sites, we conducted an additional tree ring stable isotope analysis at the SW exposed study site. The study trees selected (n=9) had similar DBH as the trees of the NE exposed study site. For a direct comparison of the beech trees analyzed for stable isotope composition, we present the mean annual DBHi of the particular nine beech trees in this study. Due to the availability of TLS images and XWF simulations, however, our study focus on the NE exposed study site and the physiological response towards drought of the respective trees (Fig.11).

Study site

The forest where all measurements and XWF simulations have been conducted is a beech-dominated stand (90% *Fagus sylvatica*) on the Swabian Alb in Southwest Germany (790 m a.s.l., 8°45'E, 47°59'N), stocking on a steep NE exposed slope (58-100%). The trees are 80 to 90 years old and the stand density is 28 m^2 ha^{-1}. The soil profile has been characterized as a shallow Rendzic Leptosol derived from limestone associated with a low water holding capacity [Geßler et al. (2005)]. The soil properties and the hydraulic parameters used for the simulation of the soil water balance can be found in Hentschel et al. (2013). The study site is located in a narrow valley. Both sites of the valley are covered by old growth beech-dominated forest and various studies have been conducted to assess the impact of differences in mesoclimate of the NE and SW exposed slope on water fluxes [Holst et al. (2010)] and stomatal conductance [Keitel et al. (2003)] of beech trees. While both study stands are comparable in structure and age, the SW exposed study site is characterized by a generally warmer and drier mesoclimate compared to the NE study site [Holst, Mayer and Schindler (2004)] and shows lower volumetric soil water contents in 2002 to 2007 [Holst et al. (2010), Tab.4]. The soil of the SW exposed study site is shallower and has less water storage capacity [Gessler et al. (2001), Gessler et al. (2004a)]. A detailed site and forest stand description of both study sites can be found in [Mayer et al. (2002), Holst, Mayer and Schindler (2004), Holst, Hauser, Kirchgäßner, Matzarakis, Mayer and Schindler (2004) and Geßler et al. (2005).

Environmental conditions

The meteorological data have been recorded at the top of a forest walk-up tower (1.5 times the stand height) located within the study stand on the NE exposed slope. The soil moisture was determined continuously on both study sites (NE and SW slope) with two probes each using the time domain reflectometry method (TDR, CS615, Campell Scientific). Detailed information on the instrumentation used for the measurements is given by Mayer et al. (2002) and Holst, Mayer and Schindler (2004), Holst et al. (2010). The drought year 2003 showed the highest seasonal values of air temperature (T; °C), global radiation (G; W m^{-2}) and vapor pressure deficit of the air (VPD; kPa) and lowest values of precipitation (P; mm) during the observation period. For the analysis of the tree ring isotope signature we also compared the NE with the SW exposed study site. The SW exposed stand is known to be more prone to drought [Holst et al. (2010)], which

Figure 11: Terrestrial laser scan image of the individual study trees at the NE exposed study site displayed in different grey shading.

is indicated by lower volumetric soil water contents (Θ; %) compared to the NE exposed site.

In order to determine different levels of drought stress, the relative extractable soil water (REW; %) was calculated by division of the actual soil water content and the maximal extractable soil water content according to Granier et al. (2007). These authors suggested a threshold of 0.4 REW when soil drought conditions start to induce stomatal regulation. The REW calculation was representative for the soil water balance model implemented in the XWF model and for the soil water simulations conducted for the upper soil layer (0-30 cm). Since the TDR soil water measurements available obtained minor data gaps, we were using the modeled soil water data for the XWF simulation and the REW calculation. The evaluation of soil water simulations resulted in a normalized root mean square error (NRMSE) ranging between 16 to 30 % for particular years (2002

to 2007), thus, providing a reliable estimate of REW over the whole growing season. Furthermore, the water deficit within the forest stand was characterized by the actual soil water deficit (Wd; mm) calculated as the cumulative difference of daily precipitation and potential evapotranspiration (pET; mm). The latter was modeled by the Penman-Monteith equation at hourly time steps according to ASCE-EWRI (2005).

Table 4: Meteorological data including air temperature (T), precipitation (P), water vapor pressure deficit (VPD), global radiation (G) and the average volumetric water content in 0-30 cm soil depth (Θ_{NE}) at the NE exposed study site and (Θ_{SW}) illustrate the average volumetric water content in 0-30 cm soil depth at the SW exposed study site. Except of P, all values are given as arithmetic mean and respective standard deviation of the growing season (01.05-30.09). P is given as sum of the growing season.

Year	T [°C]	P [mm]	VPD [kPa]	G [W/m^2]	Θ_{NE} [%]	Θ_{SW} [%]
2002	13.9(±)4.1	568	0.49	190	47(±)8	33(±)9
2003	16.9(±)5.0	258	0.88	215	37(±)1	28(±)8
2004	13.7(±)4.2	416	0.56	199	43(±)8	27(±)8
2005	14.3(±)4.5	413	0.58	195	45(±)9	32(±)8
2006	15.0(±)4.5	441	0.62	202	45(±)9	41(±)8
2007	13.8(±)3.9	414	0.37	198	49(±)7	34(±)8

XWF modeling

The IWUEXWF was determined by the ratio of the simulated assimilation A and stomatal conductance g_s. The xylem water flow (XWF) model is based on a functional-structural single tree approach with a detailed representation of the soil-leaf continuum and is implemented within in the modeling framework Expert-N 3.0 [Priesack and Bauer (2003), Priesack et al. (2007). The explicit information of the hydraulic pathway of the individual tree is used to determine the maximal water flow rates within the trees on a solid physical basis [Hacke and Sperry (2001), Schulte and Brooks (2003), Tyree et al. (1994)].

In previous studies, the XWF model has shown a good match with sap flow density measurements [Janott et al. (2011), Hentschel et al. (2013)] and is supposed to provide reliable estimates of stomatal responses. At our study site, in particular, the XWF

simulation of twelve individual beech trees resulted in a normalized root mean square error ranging between 12 and 31 % [Hentschel et al. (2013)]. The Nash-Sutcliffe model efficiency, given as dimensionless value with 1.0 indicating a perfect fit of the model (Nash and Sutcliffe, 1970), amounted on average to 0.7 at our study site and 0.75 at the study site of Bittner et al. (2012a).

The water flow in porous media, such as the xylem tissue of trees, can be described by the cohesion-tension theory [Tyree and Zimmermann (2002)] and has been calculated as a function of the gradient in water potential following Darcy's law Chuang et al. (2006). The pressure drop between the atmosphere and the soil is driven by the evaporative demand of the atmosphere and the water availability of the soil. In the XWF model, a sink/source term is added to the hydraulic pathway in order to represent the water loss of the crown due to transpiration and the water inflow due to the root water uptake and to solve the one dimensional Richards equation as suggested by Chuang et al. (2006) and Früh and Kurth (1999),

$$\frac{\delta\theta_{(\psi)}}{\delta \mathrm{t}} = \frac{\delta}{\delta \mathrm{l}}\left[\mathrm{k}(\psi)\cdot\left(\frac{\delta_{\psi}}{\delta \mathrm{t}} + \cos\alpha\right)\right] - \mathrm{S} \tag{12}$$

where θ denotes the volumetric water content (m^3 m^{-3}) at the time step t (s) for the individual cylinder element with the axial hydraulic conductivity k (mm s^{-1}) as function of the xylem water potential ψ (mm). The vertical position of the cylinder element is given by the height above (positive upward) or the depth below the soil surface (negative downward); the axial length of the element l (mm) and the respective zenith angle α (-). The sink-source term S (s^{-1}) represents the water loss of the outer branches (transpiration) and the soil water uptake by the root elements.

The aboveground tree architectures of nine trees on the NE slope were obtained by TLS conducted in the year 2011 Hentschel et al. (2013). The point clouds obtained have been further processed into a tree skeleton consistent of connected cylinders with exact position and orientation [Xu et al. (2007)]. The belowground tree architecture has been modeled according to a beech specific distribution of coarse and fine roots [Meinen et al. (2009)]. The XWF model operates at hourly basis and includes a mechanistic description of physiological response derived by the individual tree water status. A detailed description of the XWF model has been published by Janott et al. (2011), and Hentschel et al. (2013). The parameters applied for the simulations of the carbon-water balance of individual beech trees can be found in the appendix.

Stomatal conductance

The stomatal conductance g_s (mol $m^{-2}s^{-1}$) can be calculated by Fick's law in proportionality with the water vapor pressure gradient between the leaf intercellular air spaces and the ambient air,

$$g_s = \frac{E}{\delta_w} \tag{13}$$

where E is the actual transpiration per leaf area (mol $m^{-2}s^{-1}$) and δ_w is the water vapor pressure gradient (mol mol^{-1}).Under the assumption of similarity in leaf and air temperature and water vapor saturation inside the leaf, δ_w can be described by the water vapor deficit of the air [Ewers and Oren (2000)]. The stomatal conductance equation is, however, restricted to a range of environmental variables examined by Phillips and Oren (1998). Therefore, Eqn 13 can be only applied under conditions of VPD $>$ 0.1 kPa, PAR $>$ 0 μmol $m^{-2}s^{-1}$ and for time periods without rain. Following Phillips and Oren (1998), the daily mean of gs was only calculated for days with a minimum of six suitable estimates at an hourly basis.
In this study, E was simulated by the XWF model Janott et al. (2011), Hentschel et al. (2013) where the actual transpiration is controlled by the stomatal activity that is driven by the water potential at the leaf level as suggested by Bohrer et al. (2005). Calibrated on beech specific vulnerability curves (Köcher et al., 2009), the relative reduction f_S of the potential leaf transpiration due to stomatal closure is described as follows,

$$f_S = \max\left[0.1, \exp(-(\frac{-\psi}{St_b})^{St_c})\right] \tag{14}$$

where St_b (mm) and St_c (-) are curve fitting parameters and ψ (mm) is the leaf water potential. In the hydrodynamic model, each outer branch of the canopy represents the hydraulic state of the connected leaves Hentschel et al. (2013) and determines the leaf physiological control of water loss.

Photosynthesis

The XWF simulation of the actual assimilation rate at the leaf level A followed conceptually the modeling of E in a way that the potential CO_2 influx of each leaf segment is limited by g_s. This assumption was made in order to account for the diffusional restriction of carbon uptake in addition to the biochemical limitation by species-specific

maximal rates of carboxylation and electron transport [Wullschleger (1993)].
In a first step, photosynthesis was calculated according to the widely applied photosynthesis model by Farquhar et al. (1980). Here, the CO_2 assimilation rate A_{Farq} is given by the gross rates of photosynthesis determined by the Ribulose Bisphosphate Carboxylase-Oxygenase (Rubisco) activity A_c, the rate of Ribulose Biphosphate (RuP_2) regeneration A_j and the rate of CO_2 evolution from processes other than photorespiration Rd:

$$A_{\mathrm{Farq}} = \min(A_{\mathrm{c}} \mid A_{\mathrm{j}}) - \mathrm{Rd} \tag{15}$$

A summary of how the assimilation rate is modeled can be found in Leuning (1995) while the determination of Rd is described in Falge et al. (1996). A_{Farq} is calculated as a function of air temperature (K), atmospheric CO_2 concentration (mol mol^{-1}) and the intercepted photosynthetic active radiation PAR (mol $m^{-2}s^{-1}$). The parameter values applied are providedin the appendix. In a second step, A_{Farq} was corrected by multiplication with f_S (Eqn 14) representing the reduced gas-exchange at leaf level due to stomatal closure (A_{XWF}, μmol $m^{-2}s^{-1}$).

Intrinsic water use efficiency

IWUEXWF (μmol mol^{-1}) was determined by the ratio of the simulated AXWF and g_s. The seasonal average of IWUEXWF was weighted by the daily assimilation rate to estimate the amount of carbon incorporated into the tree ring archive during the growing season from May to September (IWUEw). According to dendrometer measurements by Offermann et al. (2011), however, the period from the 31st May to 10th August (day of the year (DOY) 151 to 222) has been shown most important for of beech growth. Hence, we additionally calculated IWUEw for this period (IWUEg).

Tree ring stable isotopes

The carbon and oxygen tree ring isotopic composition ($\delta^{13}C$, $\delta^{18}O$) of the nine beech trees at the NE exposed study site have been measured for the observation period from 2002 to 2007. The tree cores analysed were taken from the downhill facing side of the trunk at breast height by using an increment borer (Haglöfs, Sweden, 5.2 mm core diameter, 300 mm core depth). The sample cores have been stored in straw pipes and softly placed for transport. The cores were not sanded in order to avoid isotopic cross-

contamination over year rings, carefully dated and separated into different years under a high resolution (0.7 - 7.0) microscope (SZH 10, Olympus, Germany) by using a sharp razor blade. The samples were milled (ZM1000, Retsch, Germany) and cellulose was extracted according to Boettger et al. (2007).

Table 5: Carbon and oxygen isotopic composition (δ^{13}C, δ^{18}O) of tree ring cellulose (n=9) and oxygen isotopic composition of the altitude corrected precipitation ($\delta^{18}O_P$) of the NE exposed study site in 2002 to 2007.

Year	δ^{13}C [‰]	δ^{18}O [‰]	$\delta^{18}O_P$ [‰]
2002	-26.82(±)0.93	28.09(±)0.65	-8.89(±)2.57
2003	-26.01(±)0.91	28.71(±)0.76	-6.77(±)2.68
2004	-25.97(±)1.18	29.19(±)0.69	-7.03(±)2.59
2005	-25.90(±)1.07	28.98(±)0.65	-7.92(±)2.01
2006	-26.38(±)1.32	28.23(±)0.85	-8.06(±)3.51
2007	-26.88(±)1.49	28.03(±)0.74	-8.15(±)1.89

The determination of δ^{18}O in cellulose was performed by high temperature pyrolysis in a Flash HT elemental analyzer (ThermoFisher, Bremen, Germany) coupled to a Delta V advantage isotope ratio mass spectrometer (ThermoFisher, Bremen, Germany). The precision was < 0.2‰. For δ^{13}C measurements the samples were combusted in the elemental analyzer and the produced CO_2 was transferred to the isotope ratio mass spectrometer. The precision was < 0.1‰. The oxygen and carbon stable isotope composition was expressed using the small delta notation in permill , relative to the international Vienna Standard Mean Ocean Water (VSMOW) and the Vienna Pee Dee Belemnite (VPDB) standards, respectively. The depletion of the tree ring cellulose in ^{13}C in comparison with the CO_2 in the air (Δ^{13}C) was calculated according to Farquhar et al. (1989),

$$\Delta^{13}\mathrm{C} = \frac{\delta^{13}\mathrm{C}_{\mathrm{atmo}} - \delta^{13}\mathrm{C}_{\mathrm{cell}}}{1 + \delta^{13}\mathrm{C}_{\mathrm{cell}}} \tag{16}$$

where $\delta^{13}C_{atmo}$ represents the ratio of ^{13}C to ^{12}C in the atmosphere (-8‰) and $\delta^{13}C_{cell}$ is the carbon isotopic composition of the extracted cellulose.
In order to correct the oxygen isotope signature for the impact of changing oxygen isotope signatures of the source water we calculated the evaporative enrichment of cellulose Δ^{18}O

according to Barnard et al. (2007),

$$\Delta^{18}\mathrm{O} = \frac{\delta^{18}\mathrm{O}_{\mathrm{cell}} - \delta^{18}\mathrm{O}_{\mathrm{source}}}{1 + \delta^{18}\mathrm{O}_{\mathrm{cell}}} \tag{17}$$

where $\delta^{18}O_{cell}$ is the oxygen isotopic composition of the tree ring cellulose and $\delta^{18}O_{source}$ is the isotopic composition of average growing season precipitation. The precipitation $\delta^{18}O_P$ data was obtained from Global Networks of Isotopes in precipitation (GNIP) of the International Atomic Energy Agency (IAEA, http://$www - naweb.iaea.org$) from a GNIP station (Buchs AG, Switzerland, 380 m a.s.l.) in approx. 100 km distance. Saurer et al. (2012) showed that oxygen isotope signatures in precipitation only negligibly varied within this distance range. However, in order to determine a reliable estimate of the source water signal, we have conducted an additional altitudinal correction of the $\delta^{18}O_P$ (http://$wateriso.utah.edu/waterisotopes/pages/data_access/oipc.html$). The monthly mean values of the corrected $\delta^{18}O_P$ have been aggregated to the growing season average of the particular years (see tab.5). We are aware of the fact that the isotopic composition of precipitation is only an approximation for plant source water. Holst et al. (2010), however, showed that trees at our site take up water mainly from 10-15 cm soil depth and thus ground or aquifer water with a potentially different isotopic signature should not strongly affect the isotopic composition of the tree source water. Moreover, soil water from 10-15 cm depths is normally strongly related to the isotopic composition of precipitation water [Brandes et al. (2007)]. Differences between years might however, occur, especially when water from initial rainfall events after dry summers is locked tightly into small pores with low matric potential (Brooks et al. 2010). Depending on the water availability in the next summer, transpiration might empty these pores more or less intensively.

The rates of carbon fixation and stomatal conductance are the primary factors determining carbon isotopic discrimination and thus the intrinsic water use efficiency (IWUEiso) can be derived from $\Delta^{13}C$ according to Farquhar et al. (1982) and Seibt et al. (2008),

$$\mathrm{IWUE}_{\mathrm{iso}} = \frac{\mathrm{C_a}}{1.6}\left[\frac{\mathrm{b} - \Delta^{13}\mathrm{C}}{\mathrm{b} - \mathrm{a}}\right] \tag{18}$$

where a (4.4‰) is the carbon isotope fractionation during diffusion through the stomata, b (27‰) is the discrimination during carboxylation of Rubisco and ca is the ambient CO_2 concentration.

Because the SW exposed study stand is more prone to drought, the trees of this site were assumed to carry a stronger climate signal in the tree ring stable isotope archive. Therefore, we have included the Δ^{13}C-derived IWUE [Farquhar et al. (1982), Seibt et al. (2008)] of the SW exposed beech stand in our study (IWUEiso NE and IWUEiso SW, raw data of ^{13}C and ^{18}O are shown for the NE slope only).

Statistical analyses

Statistical analyses were carried out using R (R Development Core Team 2010). The stand variables assessed were expressed as the arithmetic mean and standard deviation of the single tree values determined by the XWF modeling and stable isotope approach. We used the nonparametric Wilcoxon rank-sum test to assess significance in differences of the tree population in varying years and in comparison between the NE and SW exposed study stands. For the ^{13}C and ^{18}O isotope values we have used a paired t-test to compare the year 2003 with the other years. For a comparison of the individual years at both study sites we used an unpaired t-test.

Results

In the following, we present seasonal (2002-2007) and intra-seasonal (daily) variations of leaf stomatal conductance (g_s, mol $m^{-2}s^{-1}$) and CO_2 assimilation rates (A_{XWF}, μmol $m^{-2}s^{-1}$) as estimated with the XWF model. As an integrated record of A_{XWF} and g_s, IWUE has been derived from the XWF simulations (IWUEXWF, IWUEw and IWUEg) and from the tree ring cellulose δ^{13}C signature results (IWUEiso). Furthermore, tree ring cellulose Δ^{18}O analyses were used to determine the stomatal sensitivity towards changing evaporative conditions. Tree growth (DBHi) has been derived from the tree ring width of the study trees and is, furthermore, substantiated by a long term chronology conducted by Poschenrieder et al. (2013). All tree data have been aggregated to the stand average based on the nine mature beech trees at each site. The occurrence of drought stress is expressed as the ratio between soil water availability and evaporative demand (REW and Wd).

Drought stress

The calculated drought indices REW and Wd indicate severe drought conditions in 2003. After the 20^{th} of July 2003, REW dropped below 0.4 (DOY 201; Fig. 12), the threshold

indicating drought stress for European forest trees as suggested by Granier et al. (2007). Wd then continuously declined to negative values down to -300 mm, indicating an increasing water deficit towards the end of the growing season. A comparable, though less intensive decline in Wd was calculated for 2006, with lowest values close to -200 mm at DOY 205 and the highest number of days with negative Wd of all examined years. In the other years examined, Wd is around zero and did not fall below -100 mm. Severe drought, as defined by REW below 0.4, has been observed only in 2003 on more than 50 days. In June, the period most important period for beech seasonal radial growth [Lebourgeois et al. (2005)], Wd did not fall below zero in the years 2002 and 2007.

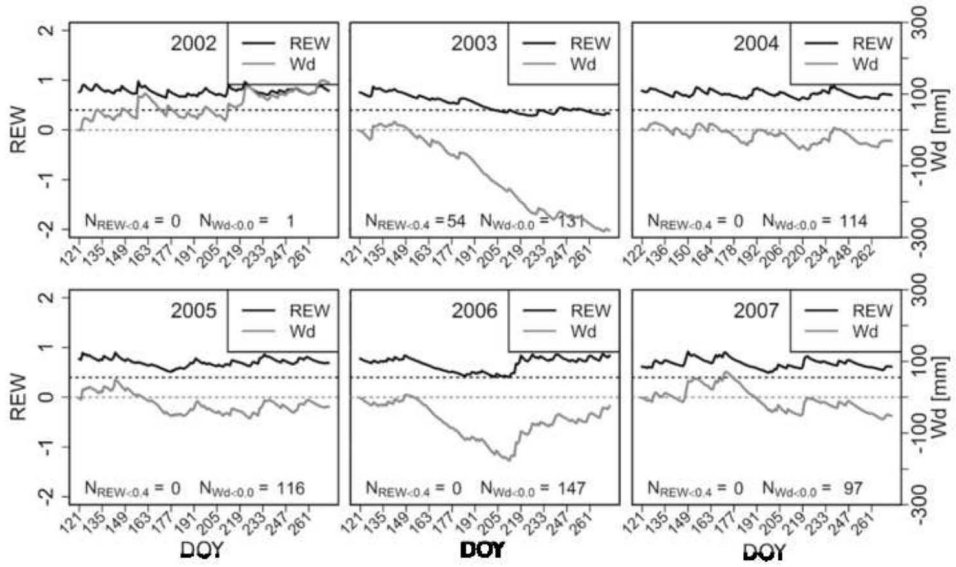

Figure 12: Relative extractable soil water (REW) in the upper soil layers (0-30 cm) and the actual soil water deficit (Wd; mm) calculated as the cumulative difference of daily precipitation and potential evapotranspiration for the growing season (May to September) of the years 2002 to 2007. NREW<0.4 and NWd<0.0 indicate the number of days below the respective threshold of water stress.

Tree growth

The mean radial growth as derived from radial growth data is shown in Fig. 13. No significant differences were apparent neither between years nor sites. At both sites, a decreasing growth trend could be observed approximately until 2005. However, while DBHi remained relatively constant at the NE site in 2006 and 2007, the DBHi increases

again at the SW site. The largest difference in DBHi between both sites (1.1 cm) was thus be observed for the year 2007.

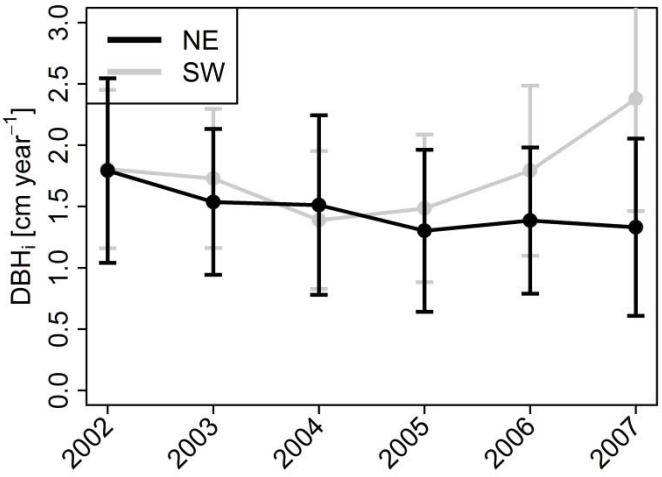

Figure 13: Mean annual radial growth (DBHi; cm y^{-1}) of nine beech trees at the NE (black) and the SW (grey) exposed study site. Dots are measured mean values and error bars denote the standard deviations (n=9).

Tree ring stable isotopes

Figure 14 shows the time series of $\Delta^{13}C$ and $\Delta^{18}O$ in tree ring cellulose of the NE exposed site (2002 to 2007). $\Delta^{18}O$ has been corrected for the inter-annual variation of $\delta^{18}O$ in source water given by the seasonal average of $\delta^{18}O_P$ (see appendix). The altitude correction between Buchs AG, Switzerland (380 m a.s.l.) and the present study site (790 m a.s.l.) resulted in a -0.6‰ difference. The tree ring $\delta^{18}O$ signature in 2003 was significantly lower compared to the other years of the time series (Fig. 14). No significant differences between the years have been observed in the tree ring $\Delta^{13}C$ signature. The mean tree ring isotope values of the nine examined beech trees varied from 18.4 (in 2005) to 19.4‰ (in 2007) for $\Delta^{13}C$ and from 35.7 (in 2003) to 37.3‰ (in 2002) for $\Delta^{18}O$. The mean standard deviation for the whole time series was 1.18‰ for $\Delta^{13}C$ and 0.73‰ for $\Delta^{18}O$. The lowest ^{18}O enrichment in tree ring cellulose has been observed in 2003, when the source water oxygen isotopic signature ($\delta^{18}O_P$) was lowest (-6.77±2.68‰, see tab. 5). Highest values in tree ring $\Delta^{18}O$ occured in 2002 and 2005. The $\Delta^{13}C$ isotopic signature in tree ring cellulose was highest in 2002 and 2007 and stayed approximately constant from 2003 to 2005 at a slightly lower level.

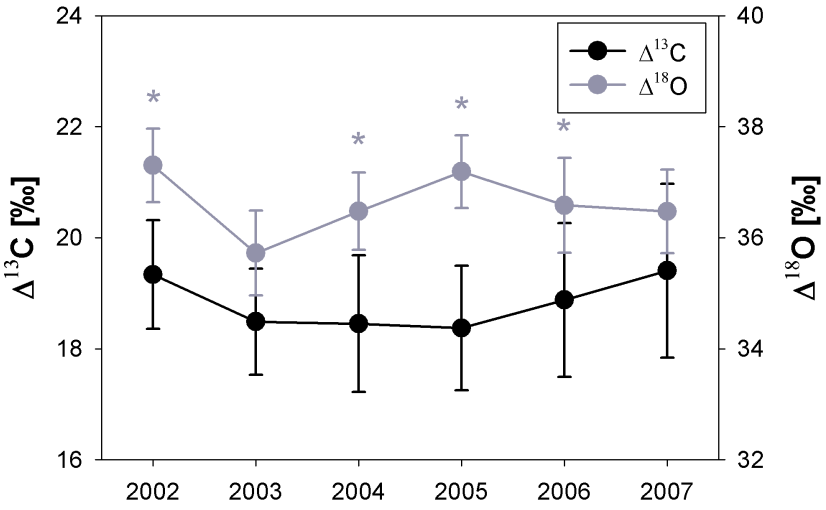

Figure 14: Carbon isotopic discrimination and oxygen isotopic enrichment of tree ring cellulose at the NE site in 2002 to 2007. The tree ring isotopic discrimination of carbon (Δ^{13}C) is indicated by the black line and Δ^{18}O is depicted by a grey line. Dots are mean values and error bars denote the standard deviations (n=9). The grey stars denote significant differences ($p \leq 0.05$) between Δ^{18}O in 2003 compared to the other years.

XWF modeling

The intra-seasonal H_2O and CO_2 exchange in the examined beech forest (NE site) for the growing season (May to September) of the years 2002 to 2007 are shown in Fig. 15. The results are illustrated as average daily values derived from hourly XWF simulation results of nine study trees.

The panels on the left hand site show the average of the daily sum of the potential and actual stand transpiration respectively (ST_{pot} and ST_{act}) and the average of the daily mean of the stomatal conductance (g_s). ST_{pot} in 2003 was about 16 to 25% higher compared to the other years. The seasonal average of gs in 2003 was about 27 to 44% lower compared to the other years. The XWF model performed a general reduction of ST_{pot} so that ST_{act} was ranging in the seasonal sum to between 47% and 63% of ST_{pot}. The highest reduction of ST_{pot} and the lowest seasonal gs has been observed in 2003. The longest period of the daily average of g_s close to zero appeared between DOY 214

and DOY 224 of this year.
The panels on the right hand site show the mean CO_2 assimilation rate of the beech trees, derived by the bio-chemical photosynthesis model (A_{Farq}) and additionally corrected for diffusional limitation (A_{XWF}). The seasonal mean of A_{XWF} ranged between 69 and 85% of the respective seasonal mean of A_{Farq}. The intrinsic water use efficiency (IWUEXWF) shown at the bottom of the panels is given as ratio of A_{XWF} and g_s. A decrease in g_s was mirrored by an increase of IWUEXWF. The highest seasonal average of IWUEXWF occurred in 2003 (144 μmol mol^{-1}) and the lowest in 2007 (78 μmol mol^{-1}). The panels in Fig. 15 (top) show the potential and actual stand transpiration as calculated with the XWF model (ST_{pot} and ST_{act}), as well as the mean stomatal conductance of the study trees (calculated as arithmetic mean of the single tree gs simulation results based on hourly time steps). High ST_{pot} above 4 mm day^{-1} has been observed from the beginning of the observation period until DOY 235 in 2003 and between DOY 162 and DOY 208 in 2007. While ST_{act} is distinctly smaller than ST_{pot} over the whole growing season in 2003 showing maximum values around 2 mm day^{-1}, ST_{act} in 2007 reached values up to 2.9 mm day^{-1}. The overall reduction of potential transpiration is calculated to be 44% in 2003 and 26% in 2007. Accordingly, relatively high seasonal gs of 0.12 mol m^{-2}s^{-1} has been obtained in 2007 compared to a g_s value of 0.05 mol m^{-2}s^{-1} in 2003. The longest period of daily gs close to zero appeared between DOY 214 and DOY 224 in the year 2003.
The lower panels of Fig. 15 show the mean CO_2 assimilation rate of the beech trees, derived by the bio-chemical photosynthesis model (A_{Farq}) and additionally corrected for diffusional limitation (A_{XWF}). The intrinsic water use efficiency (IWUEXWF) shown at the bottom of the panels is given as ratio of A_{XWF} and g_s. In correspondence to the diffusional limitation of transpiration due to decreased g_s, a reduction of A_{Farq} by 27% in 2003 and 15% in 2007 can be determined. In consequence, the seasonal average of IWUEXWF is much higher in 2003 (144 μmol mol^{-1}) than in 2007 (74 μmol mol^{-1}).

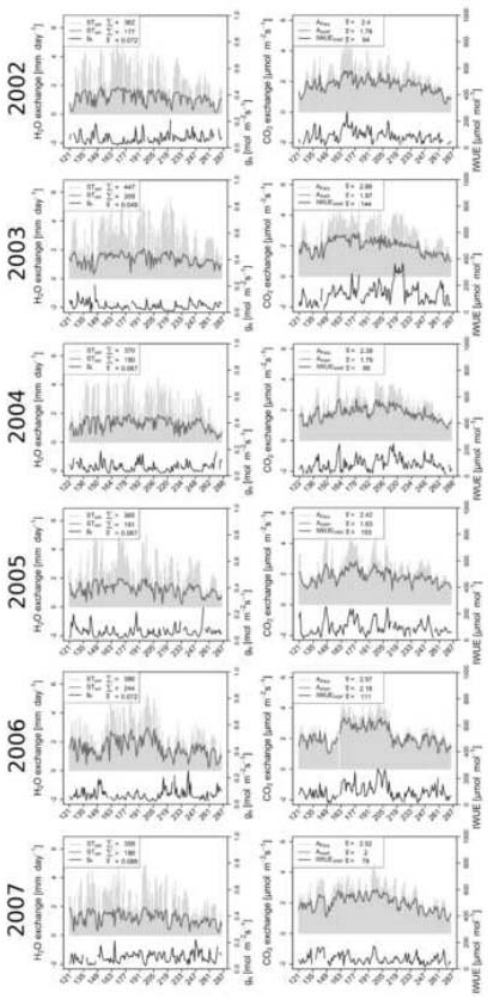

Figure 15: Seasonal courses of environmental conditions and XWF simulations for the years 2002 to 2007 as indicated at the left hand side of the figure. The panels on the left hand side show the potential and actual transpiration at stand level (ST_{pot} and ST_{act}) and stand average of the stomatal conductance at leaf level illustrated at the bottom of the panels (g_s). The panels on the right hand side show CO_2 assimilation rate derived by the Farquhar model (A_{Farq}) and A_{Farq} under consideration of the restriction of CO_2 diffusion by stomatal conductance derived by the XWF model (A_{XWF}). The intrinsic water use efficiency (IWUEXWF) was calculated as the ratio of A_{XWF} and gs and is illustrated at the bottom of the panels (IWUE). The legend of the panels show the arithmetic means ($\overline{x}$) and sums (Σ) of growing season values of the examined beech trees (n=9).

IWUE changes

IWUE derived from the tree ring cellulose carbon isotope composition (IWUEiso SW andIWUEiso NE) showed no significant differences between single years (2002 to 2007). At the SW site, characterized by a generally warmer climate and lower volumetric soil water contents between 2002 and 2007 [Holst et al. (2010)], IWUEiso SW was approx 15 μmol mol^{-1} higher than at the NE site (Fig. 16), IWUEiso NE ranges between 92 μmol mol^{-1} in the year 2003 and 81 μmol mol^{-1} in the year 2007 and WUEiso SW ranges between 106 μmol mol^{-1} in the year 2003 and 92 μmol mol^{-1} in the year 2007. The unpaired t-test revealed a significant difference between WUEiso SW and WUEiso NE in 2003. The XWF simulations of IWUE (only done for the NE slope) generally resulted in higher values compared to IWUEiso (Fig. 16). Both aggregates of IWUEXWF, weighted by the assimilation rate of the whole growing season (IWUEw, 1^{st} May to 30^{th} September) and weighted by the assimilation rate of the stem growing season of European beech (IWUEg , 31^{st} May to 10^{th} August), showed distinctly lower values compared to IWUEXWF. All estimates derived by the XWF model (IWUEXWF, IWUEw, IWUEg) indicate a significant increase in the drought year 2003. IWUEXWF decreased continuously until 2007 whereas IWUEw and IWUEg increased again in the relatively dry year 2006 (indicated by negative Wd throughout the whole growing season; Fig. 12) followed by a sharp decline in 2007. The highest agreement between the XWF simulation and the tree ring stable isotope approach was found for IWUEw with no significant differences to IWUEiso NE in the years 2002, 2004, 2005 and 2007.

Discussion

Growth response

The year 2003 has been pointed out as an extraordinary dry year associated with a reduction in gross primary productivity in several forest types in large parts of Europe, including European beech ecosystems [Ciais et al. (2005)]. Despite the generally documented growth decline of forest trees in the year 2003 [Ciais et al. (2005)] and the particular sensitivity of European beech [Granier et al. (2007)], the decline in diameter growth has not been homogenous throughout Europe. For example, no decline was observed in Northern Greece [Fotelli et al. (2009), Nahm et al. (2006)] and in the Catalonian mountains [Jump et al. (2006)]. In fact, the most severe and long-lasting declines have been observed at sites, already at the edge of the species natural distribution [Jump et al. (2006), Charru et al. (2010), Maxime and Hendrik (2011)], while the decreases in

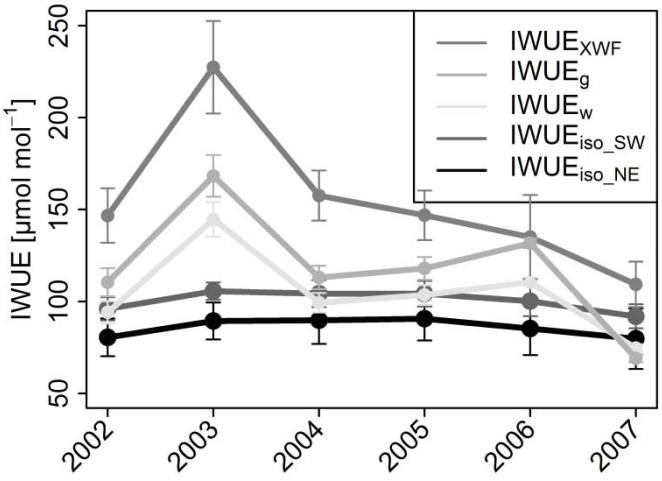

Figure 16: The intrinsic water use efficiency in μmol mol^{-1} derived from XWF simulation (IWUEXWF, grey line), weighted by the seasonal assimilation rate (IWUEw, light grey line) and calculated for the expected growing season of beech trees from DOY 151-122 (IWUEg, medium grey line). IWUEiso was derived from the tree ring carbon isotopic composition of the SW exposed (IWUEiso SW, dark grey line) and the NE exposed study stand (IWUEiso NE, black line). Note that XWF simulations correspond to IWUEiso NE. Dots are measured mean values and error bars denote the standard deviations (n=9).

other regions seem to be relatively small and short lived [Löw et al. (2006), Van der Werf et al. (2007). In other cases, only dominant trees had been considered [Weemstra et al. (2013)], which are known to be more susceptible to drought than understory trees (Pretzsch and Dieler 2011).

In this study, however, beech trees of different DBH classes and from two study sites of different soil water holding capacity did not show a significant growth response in 2003. This is against our expectations since the occurrence of drought stress has been indicated by two drought indices (Fig. 12). Furthermore, the SW exposed study site has been suggested to be generally prone to drought [Holst et al. (2010)].

Since growth decline is directly related to stress intensity [Granier et al. (2007)], the most likely explanation for the relatively small difference to other years at our site in Tuttlingen is a moderate stress occurrence in 2003. Indeed, less water depletion than in Central Germany or Eastern France beech stands has been documented at this site [Nahm et al. (2007)]. The moderate stress originates from relatively high precipitation at Tuttlingen in May as well as in July [Nahm et al. (2007)]. Although surprising, this weather pattern is not unrealistic given that the anomalously high temperatures and low

precipitation were by no means equally distributed across Central Europe [Fink et al. (2004), Rebetez et al. (2006)].
In fact, a severe drought as indicated by REW below 0.4 is only observed in late summer 2003 starting at the 20^{th} of July (DOY 201; Fig. 12). Assuming that most of the growth has already occurred before this period [Lebourgeois et al. (2005)] radial increment might not have been affected by reduced carbon assimilation due to limited soil water supply. It should also be noted that relatively warm spring temperatures result in early leaf flushing and longer overall growth periods [Capdevielle-Vargas et al. (2015)]. Such an effect has been shown for 2003 at sites in Switzerland [Leuzinger et al. (2005)] and may also apply for Tuttlingen. Finally, the high minimum temperatures in August and September as documented for Southern Europe up to Southwest Germany [Rebetez et al. (2006)] might have provoked wide latewood tree rings as has been found at beech sites in Belgium Lebourgeois et al. (2005)], masking actual biomass growth declines.
From a more general point of view, other potentially mitigating effects might also be considered. For example, Tuttlingen is exposed to relatively high ozone concentrations [Fiala et al. (2003), Solberg et al. (2008)] that might lead to a continuously decreased growth level [Grünhage et al. (2012), Subramanian et al. (2015)]. The uptake of ozone is decreased by closed stomata at low water supply which reduces growth losses by ozone and partly compensates for drought stress losses [Matyssek et al. (2006)]. Similarly, a fraction of the drought stress impact is related to a decreased nitrogen uptake [Grassi and Magnani (2005), Kreuzwieser and Gessler (2010)]. Therefore, sites with good nitrogen supply that enable a sufficient uptake are also less prone to drought stress effects. Indeed, the nitrogen supply at Tuttlingen was relatively good in 2003 [Nahm et al. (2007)].
In principle it might also be assumed that trees growing on weathered limestone might have access to deeper water from karst caves as shown for example by Jackson et al. (1999) and thus could sustain growth even when the availability of water in shallower soil layers decreases. Previous research at the same site gave, however, good indication that the trees did not have access to such deep water sources. Holst et al. (2010) compared the oxygen isotope composition of soil and xylem water at the study site and concluded that the beech trees took up water mainly from the upper 10-15 cm of the soil layer. In agreement with this finding, Gessler et al. (2001) observed that $\delta^{13}C$ in the phloem organic matter (which immediately responds to changing environmental conditions), sap flow densities and twig water potential directly responded to changes in soil water potential in the upper 60 cm of the soil profile. Thus, the measured and simulated soil water contents at our study site are suggested to represent the actual water availability and, in turn, to be a reliable indicator for the occurrence of drought stress.

In fact, drought effects on radial growth might last for several years depending on the timing of drought periods during the growing season [Eilmann and Rigling (2012)]. Several investigations have addressed the impact of the drought in 2003 on beech and observed a delayed and dampened radial growth reduction [Bréda et al. (2006), Granier et al. (2007), Skomarkova et al. (2006)]. These authors suggested that the usage of stored carbohydrates might have played a significant role for trees to cope with drought and to maintain growth which might be applied to our study. Since we observed no water limiting conditions in 2002 (Fig. 12) we assume full carbon storage prior the drought of 2003 and hypothesize that remobilization of stored carbohydrates supports radial growth for periods when carbon uptake was limited due to stomatal closure. If so, the refilling of storage C-pools might have diminished the radial growth in the following years 2004 and 2005 due to the greater sink priority of carbon storage compared to growth [Wiley and Helliker (2012)]. In fact, at the SW site, the lowest annual DBHi was observed one year after the drought (2004) what is in agreement with findings by Granier et al. (2007). In conclusion, we suggest that the lack of a significant growth response in 2003 might be explained by the seasonal timing of the drought events at the end of the growing season and by the use of storage reserves during periods of limited carbon uptake.
In fact, we observed no water limiting conditions in 2002 (Fig. 12 and Tab. 4, see also Holst et al. (2010)) and may assume well stocked carbon storage pools of the beech trees in 2003. Furthermore, Poschenrieder et al. (2013) showed a relatively high DBHi at both study sites in 2001 and 2002. Due to the greater sink priority of carbon storage compared to growth [Wiley and Helliker (2012)] this may also indicate a well stocked carbon storage. Since the tree ring chronology shows a thinning induced growth peaks in 1997 and a continuous decrease in DBHi until the year 2000 (Fig. 6 in Poschenrieder et al., 2013), the increase of DBHi in the following years strongly endorses this assumption. If we further on assume the usage of stored carbohydrates in the year 2003, the refilling of carbon storage pools used in 2003 might have diminished the radial growth in the following years. In fact, at the SW site, the lowest annual DBHi was observed one year after the drought (2004), which is in agreement with findings by Granier et al. (2007). However, given that the drought stress at the investigation site was less intense than at other Central European regions, it is unlikely that the lacking recovery is due to a particularly severe impact. This is underlined by the fact that beeches at other sites have performed a fast recovery in the following year [Van der Werf et al. (2007)]. In correspondence, we observe a continuous increase in DBHi at the SW exposed study site from the years 2005 to 2007.
Form the growth assessment only, the lack of a significant growth response in 2003, which

has been also shown by Poschenrieder et al. (2013) with a larger samples size, might be explained by three arguments: First, the beech trees in Tuttlingen experienced rather moderate drought stress, in particular due to the seasonal timing of the drought events at the end of the growing season. Second, the use of storage reserves during periods of limited carbon uptake supported the maintenance of growth. Third, the refilling of the carbon storage pools (at least) in 2004 may have masked a distinct growth response.

Tree ring stable isotopes

In agreement with a study of Saurer et al. (1997), we observe a significant decrease in $\Delta^{18}O$ in 2003 (Fig. 14). However, when looking at $\delta^{18}O$ of tree ring cellulose, no clear difference appeared among years (Tab. 5). The reduction of the ^{18}O enrichment in tree ring cellulose might be explained by the Peclet effect, which causes a reduction of lamina leaf water ^{18}O enrichment and thus $\Delta^{18}O$ of new assimilates with increasing transpiration [Farquhar and Lloyd (1993)]. Thus, the oxygen isotope results indicate increased transpiration rates in 2003. They might have overcompensated the effect of increased VPD, which would have led to higher leaf water evaporative enrichment. In fact, the highest ST_{pot} and with exception of 2006 the highest ST_{act} has been observed in this particular year (Fig. 15).

From carbon stable isotope analysis ($\delta^{13}C$) at the NE exposed site, no significant drought response between the year 2003 and the following years could be identified (Tab. 5). Various authors show clear responses towards changing environmental conditions [Libby et al. (1976), Francey and Farquhar (1982)], which has not been observed in our study. However, the lack of a tree ring isotope signal to drought is not uncommon in literature [Galle et al. (2010), Michelot et al. (2011), Penuelas et al. (2008)] and might have various reasons.

The seasonal timing of drought events has a great influence on the tree growth pattern and, in turn, on the stable isotope composition incorporated in the tree ring archive. In agreement with findings of Helle and Schleser (2004), we suggest that the early growing season, which was not that much prone to drought stress compared to the late summer of 2003 (Fig. 12) significantly influenced both DBHi and the tree ring stable isotope composition. Thus, the overall seasonal climate signal might not be mirrored by the whole tree ring assessment.

Furthermore, Skomarkova et al. (2006) derived from a stable isotope approach that about 10-20% of a tree-ring of European beech in spring is built from remobilized storage compounds while additional 10-20% of the tree-ring is affected by storage processes in autumn. In fact, from a multi-scale (leaf, phloem, xylem) stable isotope approach

conducted at the NE study site, Offermann et al. (2011) postulated that the deposition of carbon derived from starch (relatively enriched in ^{13}C) in spring and the gradually increasing incorporation of newly assimilated C (relatively depleted in ^{13}C) in the tree ring during the rest of the growing season, most likely prevented tree ring $\delta^{13}C$ signatures from being closely related to intra-annual variations in environmental drivers. Taking into account an early growth onset in 2003 followed by a late onset in 2004 at our study site as found by van der Maaten et al. (2013), the usage of storage products for tree ring formation has probably been higher in 2003 than in 2002 or 2004. Thus the isotopic results of 2003 are partly reflecting the conditions of 2002 while those of 2004 are including the drought responses from 2003 (Fig. 12). Indeed, several authors suggest the separation of tree rings for isotope analysis into early- and latewood [Helle and Schleser (2004), Barnard et al. (2012), Battipaglia et al. (2014), Voltas et al. (2013)] or high-resolution isotope measurements [Michelot et al. (2011), Schulze et al. (2004), Skomarkova et al. (2006)] in order to detect a stronger relationship between the environmental drivers and the physiological responses imprinted in the tree ring archive.
We suggest that the use of storage reserves and the late onset of the severe drought period have impacted the whole tree ring isotopic signature in our study diminishing the drought response. In agreement with the lack of a radial growth response towards the drought in 2003 the isotope signatures in the tree ring archive indicate that the growth conditions might not have been that much influenced by limited soil water supply as expected.

XWF modeling

The XWF simulation results, in particular the simulated stomatal closure, show a significant physiological response of individual trees towards the drought events in 2003 (Fig. 15). The most distinct drought response occurred for a 14 day period (DOY 212-225) when g_s dropped close to zero (Fig. 15a). During this period, a high evaporative demand ($ST_{pot} > 3$ mm day^{-1}) coinciding with a low soil water availability (REW $<$ 0.4) resulted in the simulation of very low leaf water potentials down to -2.7 MPa as obtained by the hydraulic maps of the individual trees (data not shown). The latter is in agreement with lowest leaf water potentials measured in sun-crown leaves of mature beech trees by Aranda et al. (2000) and Köcher et al. (2009).
The low values of the leaf water potential obtained in 2003 suggest that beech trees already operated near the point of catastrophic xylem dysfunction. For example Hacke and Sauter (1995) showed that about 50% of the vessels of European beech branches

experienced embolism at a water potential of -2.9 MPa. In fact, the critical hydraulic threshold of European beech as measured by pressure applications causing a loss of 50% of hydraulic conductivity of the xylem may range between -2.5 MPa for shade-grown branches and -3.15 MPa for sun-exposed branches respectively [Cochard et al. (2005), Lemoine et al. (2002)]. Thus, the simulated stomatal regulation can be seen as a plausible reaction to water loss in order to avoid hydraulic failure.
However, the XWF simulations showed a distinct reduction of the potential transpiration due to stomatal closure during the whole growing season and all years examined (Fig. 15). In fact, the stomatal response in absence of a strong soil drought (REW $>$ 0.4) is supposed to be a necessary leaf physiological response to avoid xylem dysfunction under the high evaporative demand and with regard to the maximal hydraulic conductance of the individual trees. In fact, Cruiziat et al. (2002) showed a 90% stomata closure in European beech at a xylem water potential of -2.0 MPa. Thus, we assume that stomatal conductance is minimal prior to reaching the specific hydraulic threshold and the occurrence of embolism. Indeed, Lemoine et al. (2002) observed an early and sufficiently fast stomatal response of European beech to water stress protecting the xylem from dysfunction. In the XWF model, however, g_s is controlled by the water potential gradient within the tree and, thus, depends on both the environmental conditions and the hydraulic properties of the tree. Hence, the parameterization of the hydraulic architecture of the trees, for example the porosity of the xylem tissue, influences the occurrence of critical water deficits within the whole-tree water relations and in turn the response of g_s.
Several mechanisms control g_s [Damour et al. (2010)] and complex feedback loops with environmental drivers and tree specific properties are involved [McDowell et al. (2008a), McDowell (2011)]. Furthermore, metabolic regulation of stomatal aperture is difficult to estimate [Buckley et al. (2003), Buckley and Mott (2013)] but certainly plays a role in order to optimize plant gas exchange, i.e. the loss of water to gain carbon [Chaves et al. (2003)]. Hence, the mechanistic determination of the variability in water and carbon balances and underlying processes is complex, and thus we summarize the main uncertainties and assumptions of the applied XWF model in the following two paragraphs.
Evidence of the mechanistic linkage between stomatal conductance and the leaf water potential was obtained by hydrodynamic modeling as shown e.g. by Bittner et al. (2012a), Bohrer et al. (2005) and Sperry et al. (2002). Therefore, the XWF model follows a hydraulic approach determining the leaf water status in dependence of the evaporative demand of the atmosphere, the soil water availability and of the hydraulic properties of the individual tree. This approach benefits from the simulation of the stomatal re-

sponse towards drought under consideration of the whole tree hydraulic conductance and a species-specific hydraulic threshold of the water conducting (xylem-) tissue [Johnson et al. (2011)]. Due to the complexity of the tree architecture [Cruiziat et al. (2002)], however, the structural and morphological features determined by TLS, root distribution modeling and beech specific parameterization of the wood properties, cannot account for the full heterogeneity of the hydraulic architecture. For example, xylem characteristics are assumed to be homogeneous within aboveground and belowground parts of the tree, which is not exactly the case [Früh and Kurth (1999)]. Other uncertainties concerning the fully representation of the hydraulic framework within the Soil-Plant-Atmosphere continuum might be added. In particular, the soil-root interface, representing the main hydraulic resistance within the hydraulic pathway of trees [Bréda et al. (1995), Kolb and Sperry (1999)], is subject to typical uncertainties inherent in the representation of the rooting system and the soil water balance. For example, the soil water availability at our study site is prone to high vertical and horizontal variability [Holst et al. (2010)]. The evaluation of the XWF model for 12 trees conducted at the same study site for the year 2007 [Hentschel et al. (2013)], however, showed good agreement with sap flow density measurements. In average, the authors obtained a normalized root mean square error of 15%. The parameterization of the XWF model for beech trees was obtained from literature sources (see appendix) and TLS imaging (Fig. 11) and has been applied without any fitting procedures. Hence, we suggest a reliable representation of the stomatal control by eq. 14 also for other years. The stomatal conductance, however, has been derived from simulated transpiration of the individual trees and VPD and might be biased when VPD was below 0.6 kPa [Ewers and Oren (2000)]. In order to obtain an adequate temporal resolution of g_s, however, a VPD down to 0.1 kPa has been considered for g_s calculations as suggested by Phillips and Oren (1998). Furthermore, we assumed a homogenous distribution of the climatic driving forces within the crown space and have not accounted for vertical pattern of VPD within the canopy [Schäfer et al. (2000)]. In addition, the vertical variations in leaf structural, chemical and photosynthetic characteristics associated with vertical variation in light availability (e.g. Ellsworth and Reich 1993) could not be considered in the simulation of whole-canopy carbon assimilation and stomatal conductance. The beech specific parameterization of the photosynthesis model [Farquhar et al. (1980)], e.g. the maximum photosynthetic capacity, was kept constant within the beech stand; however, variability of such parameters between individual beech trees was observed by others [Epron et al. (1995), Wullschleger (1993)]. Furthermore, the differentiation between sun- and shade-leaves as suggested by Thornley (2002) has not been considered since no information about particular leaf distributions was avail-

able.

Despite the many uncertainties of eco-physiological modeling mentioned in the last two paragraphs, the XWF simulations, however, shows a plausible range of A_{XWF} and g_s for beech trees corresponding to other studies at diurnal, daily, or seasonal scales [Epron et al. (1995), Keel et al. (2007), Keitel et al. (2003), Urban et al. (2014)]. Furthermore, we observed a plausible physiological response towards prevailing environmental conditions. In fact, the XWF simulations revealed a strong coupling of the water relations within the soil-leaf continuum, most obvious in 2003 when REW dropped below 0.4. Indeed, stomatal aperture is strongly coupled with soil water availability [Sperry (2000)]. As a consequence, the model predicts a reduced CO_2 assimilation rate with decreasing gs. A corresponding translation into growth, however, would suggest a distinct decrease of DBHi in 2003, which was not observed [Poschenrieder et al. (2013), van der Maaten et al. (2013)].

In contrast, Lebourgeois et al. (2005) found in a dendroclimatological study of 15 European beech stands across different French bioclimatic regions that soil water deficit at the particular sites were most predictive for the annual diameter growth and showed agreement with other investigations of beech forests in Central Europe. While XWF simulations indicated a distinctly decreased gas exchange at leaf level, the annual diameter growth was not significantly been affected by the drought of 2003, which might be explained by storage/remobilization processes and the seasonal timing of drought. From the hydrodynamic modelling results, however, we can accept our second hypothesis of a distinctly reduced H_2O and CO_2 exchange in 2003 due to an effective stomatal regulation of beech trees (ii).

Physiological response

In order to characterize the tree physiological response towards drought, we compared the results of growth assessment, the tree ring isotope analysis and the XWF modeling. As an integrative record of the carbon-water balance, we focused on the intrinsic water use efficiency and related our findings to the predicament of the loss of water to gain carbon. According to the carbon isotope signature, no significant differences in IWUEiso occurred between the years (2002 to 2007). However, the mean IWUEiso of the drier SW slope was about 14% higher than at the NE slope, thus, confirming a physiological acclimation of the beech trees to drier site conditions by increasing IWUE. In fact, various authors observed an increased IWUEiso of trees under conditions of high temperature and high water vapor pressure deficits of the air [Battipaglia et al. (2014), Härdtle et al.

(2013), Voltas et al. (2013)].
The ability to increase the IWUE is indeed a competitive advantage for plants under water limiting conditions [Richards et al. (2002)]. This may match to the earlier increase of DBHi after the drought at the SW exposed slope compared to the NE exposed slope showing decreased DBHi until 2007 (Fig. 13).
However, since water limitation generally evokes a reduction of g_s leading to lowered leaf internal CO_2 concentration and, in turn, increased $\delta^{13}C$ [Farquhar et al. (1989)], we expected a significant increase of IWUEiso in 2003. That has not been observed neither at the SW nor the NE exposed study site. As discussed above, the seasonal timing of drought and the incorporation of stored carbohydrates into the tree ring archive might have been masked the isotopic imprint. Furthermore, Granier et al. (2007) observed a simultaneous decrease in daily evapotranspiration and gross primary production, indicating rather constant IWUE.
Nevertheless, Damour et al. (2010) stressed that under conditions of drought, a shift in the A-togs ratio is most likely to occur. In fact, by expressing gs as a function of water relations, the hydraulic model simulates a shift towards increasing IWUEg and IWUEw under conditions of limited water availability (2003 and 2006; Fig. 16). This is consistent with existing theory and findings of a meta-analysis of 50 plant species exposed to mild water stress [Manzoni et al. (2011)]. We observe a strong coupling of the leaf physiological response (g_s and IWUEXWF in Fig. 15) with the soil water supply at intra-seasonal scale (REW in Fig. 12). The increase of the IWUE obtained by XWF modeling and induced by a reduction of g_s, however, is not reflected by IWUEiso. Since the decrease in gs during drought is suggested to be a necessary response in order to avoid xylem dysfunctioning the increase in IWUE may be added to the explanation of the maintenance of growth in 2003 as discussed. In fact, the mean A_{XWF} in 2003 is still higher compared to the years 2002, 2004 and 2005.
On the other hand, Grassi and Magnani (2005) showed that with increasing drought intensities, the contribution of non-stomatal limitation on light-saturated net photosynthesis increased and nearly equaled stomatal limitation. In agreement to these results, Epron et al. (1995) showed that CO_2 internal resistances in beech can limit A by approx. 30% - the same order of magnitude as stomata resistance. Indeed, evidence was found that the mesophyll conductance represents the main component of non-stomatal limitation of A [Grassi and Magnani (2005)] and that the balance between mesophyll and stomatal conductance is reflected in changes in leaf-level intrinsic water-use efficiency [Flexas et al. (2013)]. Furthermore, the mesophyll conductance is highly variable within the species (e.g. height, age) in response to environmental conditions and changes even

faster than stomatal conductance [Flexas et al. (2008), Han (2011), Douthe et al. (2012), Hommel et al. (2014)]. Indeed, Aranda et al. (2012) observed an decrease in both, stomatal and mesophyll conductance of beech seedlings faced to mild drought stress. Hence, we cannot exclude an overestimation of A_{XWF} in our study because non-stomatal limitations have been neglected and, in turn, IWUEXWF has been overestimated.
However, in particular in the mid- and late-season of 2003, we observed very dry environmental conditions and the simulated decrease in g_s can be seen as most likely. Thus, a physiological acclimation towards the observed drought conditions by an increased IWUE is suggested as most plausible. Furthermore, we suggest that the seasonal timing of the drought events and the usage of storage compounds have diminished both, the growth response (DBHi) and the isotopic signal in the tree ring archive ($\delta^{13}C$). Therefore, we might accept our third hypothesis of increasing IWUE under drought (iii) even so the tree ring isotope proxies did not record such a physiological response towards the drought of 2003..

Conclusion

Although European beech can be seen as a highly drought sensitive tree species [Gessler et al. (2004b)], the leaf physiological control and changes in root water uptake during water loss needs to be taken into consideration and might display an adaptive strength towards drier future climates. We demonstrate that the beech trees in Tuttlingen maintained growth in 2003 which can be partly explained by the occurrence of rather moderate drought stress at the study site. However, the hydrodynamic modeling approach also suggests an adjustment of the intrinsic water use efficiency during the drought events, thus, improving the ratio of carbon gain towards water loss.
Stomatal regulation together with adjustment of internal conductance is able to avoid harmful water tension within the xylem. However, the reduced gas exchange was neither reflected by a significant reduction in DBHi (Fig. 13) nor by a significant increase in IWUEiso (Fig.16). In fact, despite a significant decrease in gs in 2003, the mean seasonal A_{XWF} was higher compared to the previous and the following year (Fig. 15). Thus, we suggest that the seasonal timing of the drought in 2003, the leaf physiological adjustment and, furthermore, the usage of storage carbohydrates mitigated the growth response of the beech trees examined. The seasonal timing of the drought and the remobilization processes, in particular, may furthermore explain the lack of an imprint of the physiological response into the isotopic tree ring archive. Nevertheless, it is likely that the beech trees have operated at the hydraulic limits of the water conducting xylem tissue and that the impacts of prolonged drought in subsequent years would have affected the

growth considerably.
The study highlights the importance of acclimation to drought stress in beech trees and points out several uncertainties of its determination. To better identify the respective processes, a combination of ecological and physiological approaches including continuous tree growth assessments, gas exchange, and isotopic measurements in several parts of the plant are required. Due to the rapid reaction of stomatal and non-stomatal limitation of gas exchange [Flexas et al. (2008)], measurements in high temporal resolution would be desirable. Furthermore, mechanistic modeling and estimation of beech vulnerability to drought would benefit from a tighter coupling between biochemical and hydraulic approaches as well as a better resolution of physiological and anatomical crown properties. For representing growth response, we particularly emphasize the consideration of dynamic carbon storages in the plant allocation scheme.

Synthesis

In this chapter, I synthesize the results of the different projects (chapter I-III) and suggest ideas for complementary future research approaches.
On the basis of the main research question "How does drought impact the interrelated carbon and water balances in a beech-dominated forest considering different scales, from the leaf to the stand scale?", the observed findings are discussed for the European beech.

Leaf Level

Moderate soil water restriction reversibly induces plants to regulate gas exchange through their stomata. We exposed seedlings to a moderate drought scenario, which is expected to occur in the forest understory where extreme conditions (particularly humidity and temperature) are usually buffered by the overstory canopy (e.g. through shading) [Fotelli et al. (2003)]. Therefore, moderate soil water restriction realistically depicts a potential drought scenario in a natural habitat.
Diverse responses of stomatal conductance (g_s) are illustrated for understory species as well as broad leaf trees. In my study, all species except beech show reduced carbon assimilation (A), transpiration (E) and g_s, but not obligatory to the same extent. However, the extent of reduction lies within a broad range, so that WUE or iWUE can increase, decrease or remain constant during drought. This means, that a decrease in g_s not only reduces water losses, but also results in simultaneous reductions in assimilation. Reduced g_s indeed can result in an improved iWUE, on the other hand this mechanism can result in decreased assimilation. In chapter I, WUE, iWUE, tortuosity for water movement (L) and mesophyll conductance (g_m) are shown to be highly variable among the investigated species under changing environmental conditions (e.g. moderate soil drought). Various studies showed that increasing iWUE while maintaining or increasing yield would require an increase of A at any given value of g_s [see for a review Flexas et al. (2013)]. Flexas et al. (2013) showed that a simultaneous increase of A and WUE during drought is only possible in very few cases. Moreover, carbon uptake in plants is not only controlled and limited by g_s, but also regulated by the variation of g_m [Flexas et al. (2012)]. g_m represents the CO_2 diffusion from intercellular airspace within a leaf to the sites of carboxylation within chloroplasts [Caemmerer and Evans (1991)]. In agreement with Flexas et al. (2013) I found that beech respond to drought with a slight increase of WUE_{max} and $iWUE_{max}$, simultaneously observed a decrease of g_m. The increase in WUE_{max} and $iWUE_{max}$ could be explained by a higher A_{max}, a slight decrease in E_{max}

and constant $g_{s\ max}$ (see Fig.17). Flexas et al. (2015) showed that high assimilation is often associated with low WUE, due to significant correlation between diffusional g_s, g_m and photosynthetic capacities of leaves. In contrast to the findings of Flexas et al. (2015) and my studies, Cano et al. (2014) found a higher g_m linked with a higher iWUE in Eucalyptus trees. A potential factor influencing the responses of g_m is related to

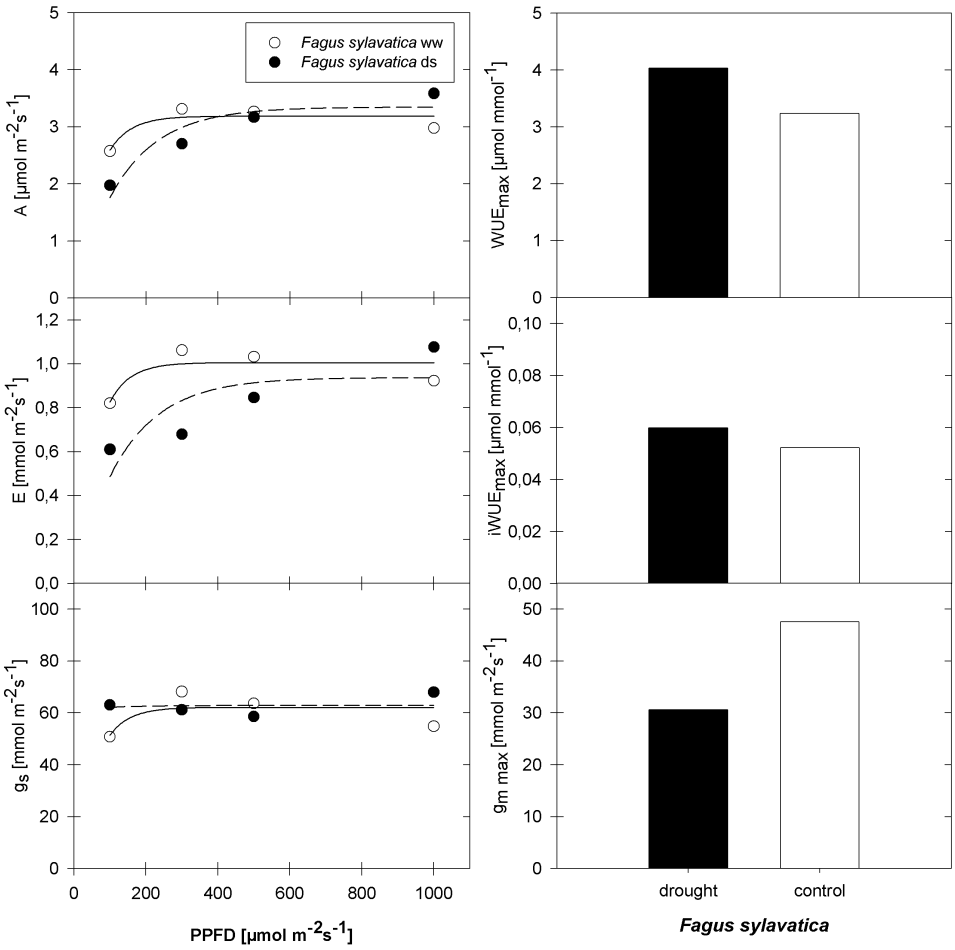

Figure 17: Light response of photosynthesis (A), transpiration (E) and stomatal conductance for H_2O (g_s) in control and drought treatments, and the calculated maximum water-use efficiency (WUE_{max})[μmol mmol^{-1}], intrinsic WUE ($iWUE_{max}$)[μmol mmol^{-1}], and $g_{m\ max}$[mmol m^{-2}s^{-1}].

leaf hydraulics. Drought can change leaf hydraulic properties and thus the tortuosity for water movement through the mesophyll [Ferrio et al. (2009), Ferrio et al. (2012)]. I showed that there were no clear correlations between g_m for CO_2 conductance and the

tortuosity of water movement in the leaf across five species in the control and drought treatments (see chapter I). In addition, I used already calculated L data from Ferrio et al. (2009), in order to verify my findings for beech. I added these L values of beech according to the study of Ferrio et al. (2009) and combined them with an unpublished dataset of mine (see appendix), including g_m of beech (see Fig. 18B for *Fagus sylvatica*). The L data from Ferrio et al. (2009) could be used, because the same experimental setup existed.

Figure 18 shows data of L, E and g_m from a wide range of different species. It clearly indicates that my results (see Fig.18A) fit well with other literature data [e.g. Song et al. (2013)]. With respect to European beech, the main finding is that $g_{m\ max}$ is reduced ($g_{m\ max}$ control = 47.6 mmol $m^{-2}s^{-1}$, $g_{m\ max}$ drought = 30.6 mmol $m^{-2}s^{-1}$) L increased and E decreased during drought (see Fig. 18B). Regarding the calculation uncertainties of L, I used different modelling approaches according to Ferrio et al. (2009). Ferrio et al. (2009) applied different evaporative enrichment modelling approaches (e.g. steady state and non-steady state) in order to predict Δ_e, Δ_{Lsp} and Δ_{Lnp} for a more precise and reliable deriving of L. L and thus leaf hydraulics (e.g. transpiration) showed consistent patterns across all species, whereas no clear response of $g_m$to L could be observed (see Fig. 18A). In agreement to Ferrio et al. (2009) and Ferrio et al. (2012), a steady increase of L during drought was recorded in my study for each species, indicating reduced leaf hydraulic conductance. The above mentioned authors measured diel courses of $\Delta^{18}O_L$ under well-watered conditions as well as soil water restriction in beech and grape vine plants. These authors, could show that L was negatively correlated to leaf hydraulic conductance. Furthermore, a correlation was also found between L and temperature [Caemmerer and Evans (2015)]. Caemmerer and Evans (2015) also observed strong differences in g_m among nine species with increasing temperature. These authors hypothesized that the observed g_m responses might have several reasons, e.g. variation in activation energy for membrane CO_2 permeability or differences in effective pathlength for liquid phase diffusion. In the mesophyll, water and CO_2 are partly transported simultaneously [Evans et al. (2009)]. For this reason, I assessed to what extent the regulation of water movement in the leaf mesophyll (modulated by the scaled effective pathlength L) influenced g_m for CO_2 diffusion during drought. There seems to be no clear relationship between L and $g_{m\ max}$ across all examined species. An explanation could be that the transmembrane diffusion of water does not play a large role for water flux in the selected species during moderate soil water restriction. If water was mainly transported via the apoplastic pathway, any change in the permeability of membranes would neither affect the effective pathlength L nor hydraulic conductivity. Another reason could be

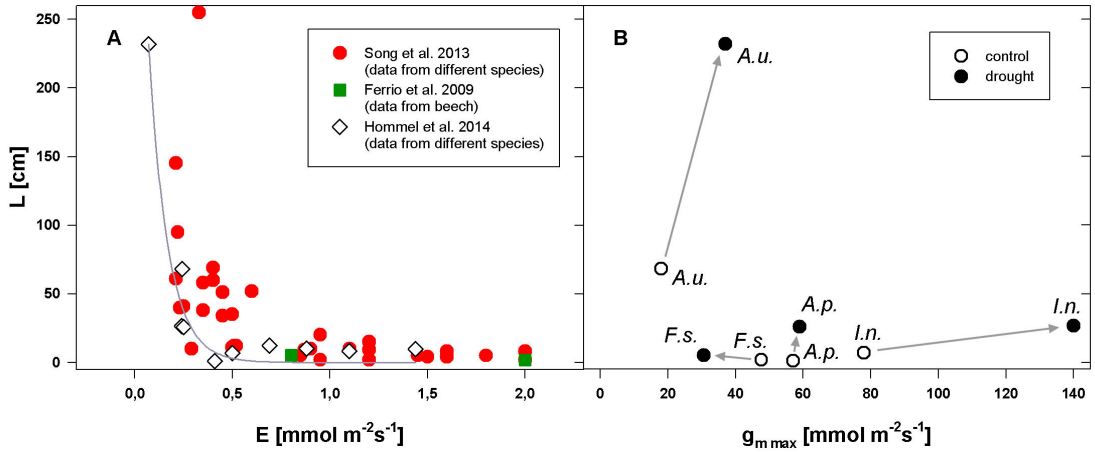

Figure 18: Relationship between the scaled effective path length L and A) transpiration at 15:00 h and B) $g_{m\ max}$ of different species. A) The black diamond symbols are data (mean values for species in a given treatment) from my study (see chapter I). A first order exponential decay function (gray line) has been fitted to the data (R^2=0.98). The red and green symbols indicate data from different species published by Song et al. (2013) and Ferrio et al. (2009), respectively. B) White and black circle symbols denote *Impatines noli tangere (I.n.)*, *Allium ursinum (A.u.)* and *Acer platanoides (A.p.)* as well as the combined unpublished dataset for *Fagus sylvatica (F.s.)*. White circle symbols are control, black circle symbols represents drought. They gray arrow denote the plant physiological reaction (direction) during drought

that the depended aquaporin-mediated CO_2 and H_2O transport suggested by Otto et al. (2010) are independent of - or even competing with each other (see Fig, 19). Aquaporins are integral tetramer proteins which form pores in the membrane of biological cells and transport material. It is hypothesized that aquaporins, which facilitate the diffusion of CO_2 through cell membranes, are involved in short-term changes of g_m [Flexas et al. (2006), Miyazawa et al. (2008), Uehlein and Kaldenhoff (2008), Evans et al. (2009)]. *"'It is not known whether this means that internal CO_2 diffusion in tissues of early land plant forms depends on simple diffusion through membranes without facilitating agents such as aquaporins"'* cited by Flexas et al. (2012) page 72 paragraph 3. Flexas et al. (2012) gave a mechanistic explanation for the fact that both assumptions are possible. (1) increasing CO_2 conductance and declining water conductance and (2) increasing CO_2 and water conductance. In their experiments they observed enhanced CO_2 conductance and declining water conductance (which means an increase in L), as well as increasing CO_2 and water conductance. I was able to observe an increasing in CO_2 transport linked

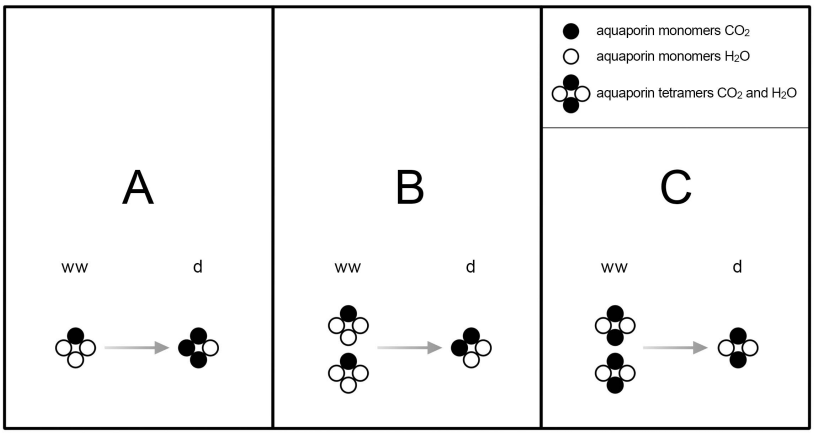

Figure 19: Expression of aquaporin tetramers and possible relationships between g_m and L for the trajectory well watered (ww) to drought (d) conditions in this study. A) increase of aquaporin monomers for CO_2 transport, decrease of aquaporin monomers for H_2O transport; B) aquaporin monomer for CO_2 transport are constant, decrease of aquaporin monomers for H_2O, decrease in aquaporin abundance; C) no change in aquaporin expression, decrease in aquaporin abundance.

to a declining water transport (case A in Fig. 19) only for *I.n.* during drought (Fig. 18B). Flexas et al. (2012) assumed that positive correlations between g_m and the path length of mesophyll water transfer were due to altered expression of a distinct aquaporin class, changing the proportion of the monomer types in the tetramers. In other words, an increase in the relative proportion of the PIP1 aquaporin facilitating CO_2 transport would increase g_m and at the same time reduce the membrane permeability for water. Negative correlations between g_m and L might be due to variations in total aquaporin abundance with no changes in the composition of the tetramers (see Fig. 19C). Accordingly, a simultaneous increase of g_m and L during drought was evident observed only for the species *Impatiens noli tangere* as mentioned above. In the other species either a negative relationship could be observed (see Fig. 18B, 19C *F. sylvatica*), *F. excelsior* and *M. annua*) or an increase of L with no change in g_m (*A. platanoides*) was detected (see Fig. 18B, 19B). Therefore, I assume that the regulation of aquaporin expression and tetramer composition differs among species, which could lead to the observed lack of consistency in the relation between g_m and L.

My results indicate that an increasing g_m does not automatically imply a reduction of L, and thus a decreasing in the tortuosity of the water movement through the mesophyll , or vice versa. Therefore, I have to reject my working hypothesis in objective **1**.

A higher number of investigated plant species (n>40) could help to get a clearer correlation between g_m for CO_2 and water conductance during drought. However, the problem of a proper classification system for different "soil moisture levels" remains, which is so far not sufficiently defined in the literature. In future studies it could be helpful to apply gradients of drought levels to obtain threshold values or clear defined pedotransfer functions, in order to classify the dimension of plant vulnerability or target for breeding and genetic engineering [Cano et al. (2014)]. Classifications on the basis of volumetric or gravimetric water content, pore space, soil moisture tension and -potential or a qualitative comparison of soil drought do not sufficiently take into account the different susceptibility or tolerance of the different plant species and do further not give a reliable estimate of plant-available water, which restricts the comparability between studies. Drought thresholds are related to the water availability over several months and do not necessarily reflect single drought events in terms of their intensity and duration (e.g. modeled REW according to Granier et al. (2007) or derived DSD according to Zang et al. (2014)). Moreover, I postulate that more severe soil drought, as applied by Ferrio et al. (2012), might cause a stronger interference between the pathways of water and CO_2 movement, and that inter-specific variation or differences between functional plant groups (e.g. light- vs. dark-adapted species) might determine the degree of interference. An additional help to classify the plant status (e.g. vitality during drought) quickly and preventive, could be a standardized measurement of proline concentrations at the leaf level [Fariduddin et al. (2009)]. The combination of such biochemical biomarkers and the above mentioned threshold functions would improve the comparison of the different studies.

In future studies, the focus should be on species which already expressed an increasing of g_m. Thereby it is necessary to verify if variations in g_m are accompanied by variations of plant specific aquaporin content and also which aquaporin is responsible for the observed change in g_m, under varying environmental conditions [Steudle and Frensch (1996), Martre et al. (2002), Terashima and Ono (2002), Sack et al. (2004), Flexas et al. (2006), Cochard et al. (2007), Miyazawa et al. (2008), Uehlein and Kaldenhoff (2008), Evans et al. (2009), Heinen et al. (2009), Otto et al. (2010), Kaldenhoff (2012), Pou et al. (2013), Caemmerer and Evans (2015)]. A promising approach to test which aquaporin is responsible for observed changes in g_m or hydraulic conductance, is the application of proteomics analysis and to interpret and implement the results in isotope model approaches.

For a better understanding of g_m, Flexas and Diaz-Espejo (2014) recommended to apply structural models including interactions in cell walls, in order to get a better knowledge

of the physico-chemical structure of cell wall pores and their effects on water and CO_2 diffusion. These authors further suggested to consider chloroplast morphology and distribution properties (and to add these to the models). For example, it remains to be proven whether cells and chloroplasts shrink in response to changing environmental conditions and linked with an increase of VPD. Nevertheless, in the present study I observed that WUE, iWUE and g_m of beech seedlings were only slightly affected by exposure to moderate drought. Therefore, I conclude that beech seedlings are mainly tolerant to moderate drought at the leaf level. Theses findings also have implications for the transfer of biochemical information from leaf level measurements to the canopy and stand level by using photosynthesis models [Flexas and Diaz-Espejo (2014)].
I recommend to carry out field studies with mature trees in order to compare the results with my findings (see chapter I). A complementary research approach could be to assess how g_m of mature beech trees is affected by tree height or age, when exposed to drought and competition, and if it plays a role in improving WUE or iWUE in a given age or height class. For example, Han (2011)) stated a negative correlation between g_m and tree height for *Pinus densiflora*. The author presumed that the height related decrease in photosynthetic activity is mainly influenced by diffusive resistances rather than biochemical limitations. Thus, it would be conceivable that the identified differences at the forest stand level, i.e. mismatch between modeled iWUE (IWUEw) and isotope based iWUE (IWUEiso) of the mature trees, could be explained by changing in g_m during the drought in 2003 (see chapter III, Fig. 16). In contrast, Cano et al. (2013) observed a lower g_m in lower compared to upper canopy leaves of beech and oak. In addition, Cano et al. (2013) and Douthe et al. (2012)) found that g_m responded within minutes (reduced by 60%) when irradiation switched from 600 to 200 μmol $m^{-2}s^{-1}$. Hence, the assessment of responses of mesophyll conductance for CO_2 with higher resolution than that achieved with chlorophyll fluorencence measurements is desirable and necessary. An increase of the temporal resolution would greatly improve the explanatory power of the results and help to derive more precise complementary research approaches on the leaf level (e.g. to quantify the variation of g_m during a day in combination with L).

Tree Level

Pulse labeling of trees with ^{13}C enriched or depleted CO_2 facilitates tracing the pathway of newly built assimilates within different plant organs, e.g. to determine transport velocity or allocation patterns (storage or remobilization), and their release into the soil. The objective of chapter II was to assess these patterns for two different tree species (*Fa-*

gus sylavatica and *Acer Platanoides*) during drought, considering inter- and intraspecific competition, by applying ^{13}C pulse labelling. Drought appears to result in delayed and reduced carbon transfer within the plant and from the plant into the soil [Ruehr et al. (2009), Barthel et al. (2011)]. In a review based on 47 studies, Epron et al. (2012) summarized the whole-tree C source–sink relations, C allocation to secondary metabolism, responses to environmental change and effects of seasonality versus phenology in different plant tissues. But how is the carbon allocation affected by interspecific competition plus drought? Until now no study has been performed to trace labelled assimilates under such conditions, and thus the study in chapter II fills an important gap.

Figure 20 depicts the onset of ^{13}C-enrichment of CO_2 respired in the soil surrounding the roots of different ^{13}C-labelled tree species (including data from studies of Epron et al. (2012) and Studer et al. (2014) without interspecific competition and drought (A) and during drought also without interspecific competition exposure (only beech; B), plotted against tree height. Even though the data are strongly scattered there is a weak linear relationship between tree height and the time of the first label appearance (see Fig. 20). The variability in the different studies could e.g. be caused by differences in soil moisture, drought duration, irradiance, nutrient availability or provenance. Figure 20 indicates that drought generally results in a delayed carbon transfer from the plant to the soil interface. Furthermore, it seems that the velocity of carbon transport to the roots (mostly influenced by significant differences in g_s and assimilation) during drought is positively correlated to the tree height. The moderate drought treatment, as applied in the study of chapter II, slightly increased the transport of new assimilates in beech compared to the control, but no significant reduction of transport velocity occurred. However, the experiments in chapter II show that interspecific competition affects assimilate allocation within trees to different plant organs (e.g. roots) even more than the exposure to moderate drought. The beech moderate drought plus competition treatment forced even more the relative allocation of new assimilate belowground. Therefore, I also have to reject my working hypothesis in objective **2**.

In contrast to my initial hypothesis, drought increased carbon allocation within the plant and enhanced the coupling between the above- and belowground plant system. The increased transport might be important to maintain the functioning of the root system, since reduced water availability increases fine root mortality and also stimulates compensatory fine root production [Meier and Leuschner (2008b)]. Chapter II points out that beech root biomass significantly differed between monoculture (intraspecific competition) and mixed treatment (interspecific competition), but not within a treatment (drought and control). It further should be mentioned that the effective vertical

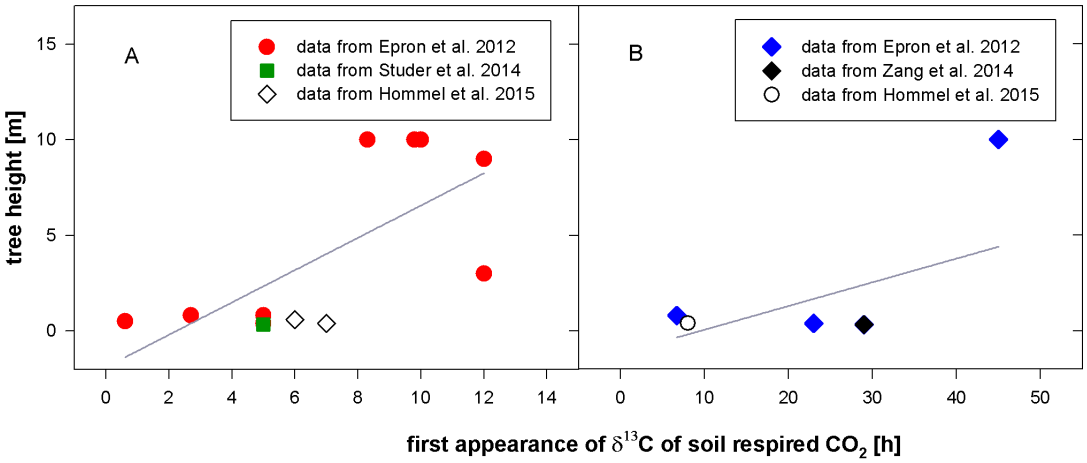

Figure 20: Relationship between the time [h] until the appearance of ^{13}C of soil-respired CO_2 after labeling and tree height [m]. A) Control treatment; black diamonds depict beech and maple control plus intraspecific competition from my study, red and green symbols represent different tree species taken from Epron et al. (2012) and Studer et al. (2014), respectively. A linear regression (gray line) has been fitted to all data (R^2=0.48). B) Drought treatment; blue and black diamond symbols show beech data taken from the review of Epron et al. (2012) and the study of Zang et al. (2014), respectively; the white circle indicates beech drought plus intraspecific competition data from the study in chapter II. A linear regression (gray line) has been fitted to all data (R^2=0.28).

space within the tubs was limited (Unfortunately, this effect was noticed only after the harvests), thus very likely influencing the root development and growth. However, such restrictions might also occur in the field in shallow soils Fotelli et al. (2003). Additionally, missing discrimination between dead and intact roots might have led to biased estimates of root biomass. It is also assumed that a decoupling arises between canopy layer (leaf) and belowground compartments (roots) only during serve drought events [Offermann et al. (2011), Pflug et al. (2015)].

Furthermore, $\delta^{13}C$-enrichment of the respired CO_2 in the beech drought plus competition treatment ("mixed treatment") after 2 days was significantly lower compared to the control mixed treatment. A 2.9 fold higher MRT upon drought was accompanied by an increase in $\delta^{13}C$ in root WSOM. An explanation could be that beech accumulates sugars as osmoprotectants to raise osmotic pressure, maintain membrane integrity and stabilize proteins [Peuke et al. (2006)].

The summary of chapter II is that drought reduces A in beech and maple, with a stronger

effect in beech. Both species maintained their transport of new assimilates to the roots and in the monoculture (intraspecific competition), beech exposed to moderate drought allocated more newly built assimilates to the roots than during unlimited water supply. This pattern was even pronounced under interspecific competition, which was shown to have huge influences on carbon allocation dynamics in beech and maple seedlings. Thus, juvenile-beech trees are still able to efficiently allocate new assimilates belowground during drought, even under additional inter-specific competition. This might be seen as a long-term strategy to maintain the root osmotic potential and function. For further research I suggest to investigate phloem cell size in order to derive phloem loading and transport strategies. With regard to the long term changes in the carbon source-sink relation, I recommend to carry out longer competition experiments (e.g. >10y) like http://*www.treedivnet.ugent.be/ExpBIOTREE.html*.. The BIOTREE experiment in has been established in 2004 and deals with effects of altered species richness and functional diversity characteristics and processes, including long term C-source sink relations. Important information of plant water uptake during competition benefits from longer observation periods was provided by assessment of [Grossiord et al. (2014)]. Furthermore, the simulation of longer drought periods (i.e. several years) promises valuable insights into the diverse mechanisms determining the stress resilience of trees [Gimbel et al. (2015), Felsmann et al. (2015)]. Gimbel et al. (2015) reduced precipitation about 26-33% over two years in order to assess long term moderate drought effect. In addition, the timing of the drought period (e.g. spring, summer or at the end of the growth period) might be important and needs to be considered in future studies. Different drought treatments could be applied during, before or after the major growth period of the trees. However, it should be noted that the present study only dealt with drought impacts. Including rewetting effects would further help to improve our understanding of allocation, growth, storage and remobilization patterns in trees and to assess their resilience and recovery potentials in the more variable climate of the future [Zang et al. (2014), Baudis et al. (2015)].

Stand Level

Drought has considerable impacts on the water and carbon interactions and change their relations in trees [Lieth (1973), Gholz et al. (1990), Scurlock and Olson (2002), Huxman et al. (2004), Ciais et al. (2005), Granier et al. (2007)]. What are the consequences at the stand level? When scaling up to the stand level, I found a reduction of water use and leaf water potential with increasing drought. Moreover, the hydraulic conductance

from the soil-root interface to the leaves decreased. The soil to leaf water transfer has been approached in models by Sperry et al. (2002). These authors linked hydraulic architecture in tress and soil resistances. Besides, the authors observed strong differences among species in water use mainly due to their different hydraulic properties. Moreover, numerous studies describe the vulnerability of cavitation and hydraulic architecture of tree species [Tyree and Zimmermann (2002), Bohrer et al. (2005), Chuang et al. (2006)]. Chapter III highlights the importance of acclimation of beech trees to drought stress and points out several uncertainties. Terrestrial laser scans (TLS) are incorporated into a xylem water flow model (XWF), in order to derive more precise plant physiological reactions (e.g. stomata closure) towards changing environmental conditions. The linkage between TLS and the XWF model allows an improved characterization of the entire aboveground tree architecture [Bittner et al. (2012), Hentschel et al. (2013)]. The objective of chapter III was to compare the iWUE derived from the XWF model with the iWUE derived from δ^{13}C of cellulose extracted from tree cores of mature beech trees.
I could not find any significant difference in mean radial growth and derived iWUE (based on the isotopes measurements) between the investigated years (2002-2007) I did not find significant differences between the years of investigation (2002-2007). In contrast, growing season integrated iWUE derived from the XWF-model approach showed a clear peak in 2003. Therefore, my third hypothesis assuming increasing iWUE under drought conditions can't be accepted with respect to the isotopic results. The discrepancy between the measured (i.e. isotope-based) and modeled values could possibly be lowered by the inclusion of complex remobilization processes of NSC into the XWF model. NSC sustains the metabolic processes of the cambium, including tree ring growth. At the beginning of the growing season, NSC concentrations increase and peak when resource demand is highest [Gessler et al. (2014)]. A high demand occurs when the main part of xylem cells are in phases of enlargement and cell wall thickening [Simard et al. (2013)]. Thus, the high seasonal demand for NSC is in spring likely causes the remobilization of stored carbohydrates from previous years, especially in deciduous species, and thus mixes the substrates used for cellulose biosynthesis. In that δ^{13}C in tree rings gets partially uncoupled from that of new assimilates [Helle and Schleser (2004)]. As a consequence, the iWUE values calculated with the XWF model will not be reflected by the tree ring δ^{13}C values.
According to Gessler et al. (2014), it is also possible that the δ^{13}C isotope signal in the tree ring cellulose is influenced by other isotope mixing processes e.g. diel variations in δ^{13}C as a consequence of transitory starch storage and remobilization or seasonal variations of δ^{13}C during heterotrophic starch synthesis. The metabolic processes of

the cambium contribute to a change of $\delta^{13}C$ isotope signal in the tree ring cellulose, e.g. through storage, remobilization, respiration and re-fixation of NSC in heterotrophic tissues (see Fig. 21). For example, during the light period, starch is produced and

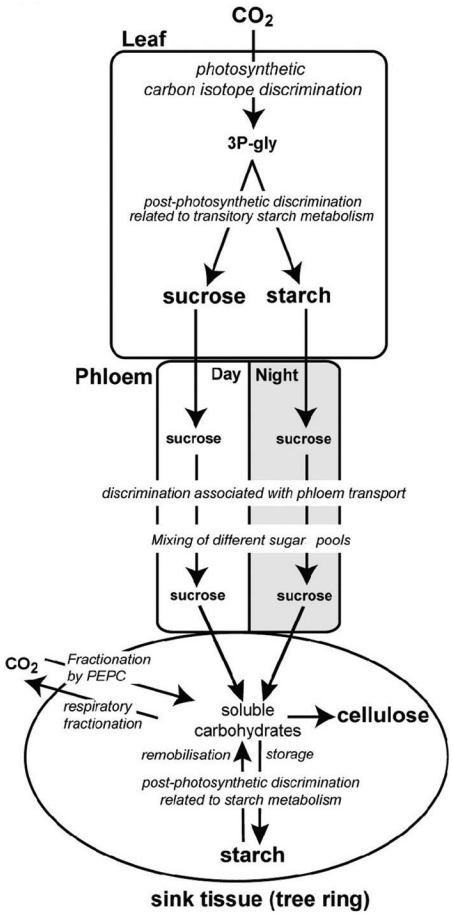

Figure 21: Overview of different processes influencing the carbon isotope signature, from primary sources CO_2 and H_2O, to tree ring cellulose, going through different organic and inorganic pools according to Gessler et al. (2014)

accumulated in the chloroplasts of leaves, and the production of sucrose in the cytoplasm involves ^{13}C-depleted triose phosphates exported from the chloroplast. This ^{13}C depletion is a direct consequence of transitory starch synthesis, which favors ^{13}C during production of fructose-1,6-bisphosphate by aldolase. Furthermore, it is very likely that seasonal variations in soil moisture determine the timing of starch accumulation and breakdown. C isotope fractionation during heterotrophic starch synthesis could be one of the reasons for the seasonal variation of $\delta^{13}C$ during periods of storage reserve remobilization within the stem, and thus uncoupling of the $\delta^{13}C$ signal in the tree ring

from environmental drivers [Gessler et al. (2014)].
Furthermore, comparable effects of storage and remobilisation occur for $\delta^{18}O$. The signal originates also partially from the stored carbohydrates during starch remobilization. Gessler et al. 2007 show that 5 out of 11 oxygen atoms in sucrose generated from starch are exchanged with the surrounding non enriched water. Thus 6 oxygen atoms and their $\delta^{18}O$ are retained from the storage compounds which cause an uncoupling between the canopy and the tree ring [Offermann et al. (2011)].
Therefore, I state that the two different methods fit not well together (with respect to objective **3**), even though the XWF showed strong impacts of inter-annual climate variations on the water and carbon balance in beech, especially in the extraordinary dry year 2003. However, the growth and iWUE parameters derived from tree rings were converse to my hypothesis in objective **3** which could therefore not be confirmed. Granier et al. (2007) used a daily water balance model in order to quantify the site specific impact of drought. These authors emphasized that the huge drought effects in 2003 were site- and species-specific. A reduction in tree growth of about 50% was observed in lowlands (e.g. Hesse, Sorø) but not in steep hill areas (e.g. Tuttlingen, Tharandt) [Ciais et al. (2005), Granier et al. (2007)]. Theses authors stated that the growth reduction was more pronounced in the year following the drought (2004).
Another reason for the lacking response of tree ring growth and isotopic composition towards the drought in 2003 in my study might be related to the limestone bedrock the forest is stocking on and many limestone formations are known to be weathered easily and contain karstic features. Therefore, they have the ability to retain large quantities of water, which might be accessed by sinker roots of beech trees. Such deep carstic water would allow the trees to take up and use water, even though the upper soil horizons are desiccated. However, this assumption has been not supported by previous studies from Gessler et al. (2001) and Holst et al. (2010) at the same site. These studies gave a good indication that the trees did not have access to karstic water resources. Gessler et al. (2001) observed that $\delta^{13}C$ in the phloem organic matter, sap flow densities and twig water potential directly responded to changes in soil water potential in the upper 60cm of the soil layer. Moreover, Holst et al. (2010) compared the oxygen isotope composition of soil and xylem water at this site and concluded that the beech tree root water uptake is mainly from the upper 15cm of the topsoil layer. Thus, the measured and simulated soil water contents at the study site are suggested to represent the actual water availability and, in turn, to be a reliable indicator for the occurrence of drought stress.
Nevertheless, the XWF model possibly overestimates the effect of drought on iWUE in 2003, by neglecting stomatal limitations. The XWF model work at leaf level scale and

does not take into account carbon storage and mobilisation processes in the trunk. As already mentioned, drought lead to an uncoupling of the carbon relations between leaf canopy layer and the trunk. Thus, the result is strongly influenced, because it is also possible that the beech trees have operated at the hydraulic limits of the xylem tissue. An improvement of our mechanistic understanding, especially the linking between canopy and trunk (e.g. uncoupling processes) is thus desirable. Thereby it is absolutely necessary to take storage and remobilization processes within the trees and soil water measurements within the root zone into account. Moreover, as mentioned above, it is also important to consider the seasonal timing (e.g. separation in early- and latewood). Another difficulty regarding the upscaling from single trees to forest stand-level is the high variability of g_s, the little knowledge about g_m of mature trees (e.g. tree height or age) and its effects on $\delta^{13}C$, thereby inevitably iWUE between the trees and within the trees (e.g. influenced by competition). Thus, not only a high sample size is required for reliable estimations on the forest stand level [Wutzler et al. (2008)], but also the knowledge of c_i and c_a time series should be taken into account. A promising approach to estimate iWUE on the forest stand level was recently given by Frank et al. (2015). These authors, compared annually resolved long term $\delta^{13}C$ tree ring measurements across Europe with the leaf level CO_2 and assimilation to g_s and c_i-c_a differences, to an dynamic global vegetation model (DGVMs) in order to quantify iWUE on the forest stand level. The advantage of this DGVMs is the consideration of fractionation processes during carboxylation and stomata diffusion. The weakness of this model is that no storage or remobilization processes are considered.

Conclusion and Prospects

In a nutshell, the vulnerability of beech and other relevant species to a single realistic soil drought event (< -0.5 MPa) in beech dominated forests is negligible at different scale levels [Blessing et al. (2015)]. As a key parameter iWUE was used in each chapter to assess plant physiological reactions, to derive different strategies with regard to drought and vulnerability. I illustrate different tendencies in g_m (chapter I) and C allocation (chapter II) during a single moderate drought event and recommended new experimental approaches to improve the simulation and the understanding of plant physiological reaction during periodically occurring drought events (including soil moisture thresholds) at the ecosystem scale. Furthermore I pointed out that in a more diverse system (i.e. competition in combination with drought) biotic parameter influencing plant physiological reactions [Grams et al. (2007)] than abiotic parameters. Thus, inter- and intraspe-

cific competition should always be considered, because it is present in every terrestrial ecosystem (see chapter II and III). Only few studies deal with whole plant and ecosystem carbon allocation (including the plant/soil interface) during drought with a high temporal resolution (Ruehr et al. (2009), Barthel et al. (2011), Zang et al. (2014), Blessing et al. (2015)]. The most promising approaches are the use of isotope laser techniques for the continuous measurement of gas flows as well as combined ion massspectrometer methods for the precise determination of compound specific label concentrations [e.g. Lehmann et al. (2015)] with high temporal and spatial resolution [e.g. Blessing et al. (2015)]. Only stable isotope tracer experiments are suitable to study ecosystem pathways as well as the fate of carbon and carbon-water interrelations [Epron et al. (2012), Grossiord et al. (2014)]. The investigation of stable isotopes (e.g. to link the partial exchange of xylem water during cellulose biosynthesis with leaf water during carbohydrate biosynthesis, mixing of sugar pools with different metabolic history during phloem transport) and the conversion of steady state models [Craig and Gordon (1965)] to more dynamic non steady-state models [Farquhar and Cernusak (2005)] or DGVMs [Frank et al. (2015)] would greatly help to improve our understanding of individual as well as global responses to drought and competition in plant physiology. Last but not least, to transfer this newly gained knowledge from leaf level to the stand level (e.g. isoscapes), a lot of research on non-linear processes at scale borders is still needed.

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Appendix

Selbstständigkeitserklärung

Hiermit erkläre ich, dass ich die vorliegende Doktorarbeit selbstständig und ausschließlich unter Verwendung der angegebenen Quellen sowie ohne unzulässige Hilfe Dritter angefertigt habe. Diese Doktoararbeit wurde bisher weder im Inland noch im Ausland in gleicher oder ähnlicher Form oder auszugsweise einer Prüfungsbehörde vorgelegt.

Berlin, den 27.11.2015

Robert Hommel

Stable isotopes in tree rings: towards a mechanistic understanding of isotope fractionation and mixing processes from the leaves to the wood

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Impact of interspecific interactions on the soil water uptake depth in a young temperate mixed species plantation

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Importance of tree height and social position for drought stress and induced mortality

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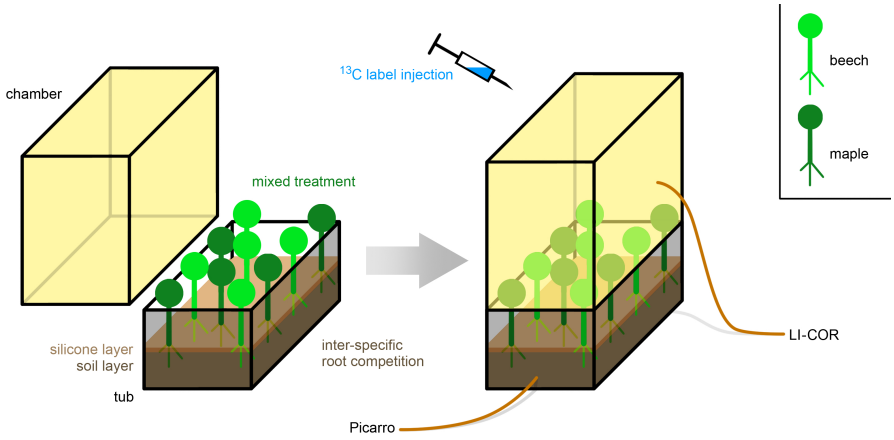

Figure 22: Schematic experimental set up of beech and maple in mixed treatment. After the labelling (2h) the chamber and the silicone layer were removed.

Table 6: Unpublished dataset of well watered beech treatment, assimilation (A) in [μmol $m^{-2}s^{-1}$], intercellular CO_2 concentration (c_i and c_c) in [μmol mol^{-1}], J in [μmol e^- $m^{-2}s^{-1}$], substrate specificity factor (S_Γ) in [mol mol^{-1}], CO_2 compensations point (Γ*) in [μmol $m^{-2}s^{-1}$], mesophyll conductance (g_m) in [mmol $m^{-2}s^{-1}$], θ_s is the average volumetric water content (±SD) during the treatment.

species	CO_2	light intensity			
parameter		1000	500	300	100
A	200	1.94±0.90	1.78±0.88	1.97±0.71	1.64±0.23
	150	1.25±0.58	1.16±0.46	1.21±0.43	1.07±0.17
	125	0.90±0.41	0.80±0.33	0.82±0.30	0.75±0.15
	100	0.41±0.26	0.31±0.20	0.44±0.36	0.25±0.11
	75	-0.25±0.20	-0.16±0.04	-0.21±0.07	-0.38±0.19
c_i	200	155±5	156±5	152±8	159±8
	150	125±2	123±4	123±4	127±4
	125	108±1	107±2	107±2	109±2
	100	91±2	92±1	94±4	93±1
	75	77±4	75±1	76±1	78±3
c_c	200	29.57	30.13	27.82	24.91
	150	22.74	23.30	18.95	16.76
	125	19.76	19.15	14.37	12.49
	100	15.56	14.06	9.98	5.86
	75	9.56	8.98	3.12	0
J	200	34.77	31.33	30.43	22.45
	150	34.02	31.00	28.05	21.45
	125	34.20	29.30	26.43	20.78
	100	33.61	28.05	24.96	19.40
	75	31.30	26.33	23.24	17.73
g_m		35.30	32.85	41.93	35.53
S_Γ	3333				
Γ*	32				
Age (y.)	2				
Origin	810 23				
Height (cm)	30-50				
θ_s (%)	51±4				

Table 7: Unpublished dataset of dry conditions beech treatment, assimilation (A) in [μmol $m^{-2}s^{-1}$], intercellular CO_2 concentration (c_i and c_c) in [μmol mol^{-1}], J in [μmol e^- $m^{-2}s^{-1}$], substrate specificity factor (S_Γ) in [mol mol^{-1}], CO_2 compensations point (Γ*) in [μmol $m^{-2}s^{-1}$], mesophyll conductance (g_m) in [mmol $m^{-2}s^{-1}$], θ_s is the average volumetric water content (±SD) during the treatment.

species	CO_2	light intensity			
parameter		1000	500	300	100
A	200	1.64±0.46	1.57±0.28	1.35±0.36	1.04±0.44
	150	1.08±0.32	0.97±0.25	0.86±0.26	0.68±0.37
	125	0.74±0.24	0.62±0.15	0.63±0.19	0.49±0.32
	100	0.28±0.21	0.21±0.05	0.23±0.13	0.14±0.27
	75	-0.14±0.11	-0.12±0.08	-0.09±0.02	-0.29±0.25
c_i	200	156±10	138±40	161±15	165±16
	150	124±5	122±6	128±8	129±10
	125	102±11	109±2	110±5	110±6
	100	92±3	93±1	93±3	95±6
	75	75±2	73±2	74±1	83±13
c_c	200	25.01	19.79	25.79	20.25
	150	19.54	12.96	19.64	15.16
	125	17.49	9.92	16.66	12.61
	100	13.62	6.36	12.06	7.90
	75	9.745	2.38	8.01	2.41
J	200	33.48	29.10	24.55	18.93
	150	28.89	24.03	22.20	18.17
	125	32.46	23.78	21.18	17.74
	100	30.54	23.15	20.36	16.66
	75	28.07	20.78	18.86	15.50
g_m		26.54	32.83	21.02	19.21
S_Γ	4178				
Γ*	25				
Age (y.)	2				
Origin	810 23				
Height (cm)	30-50				
θ_s (%)	23±2				

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